MYCOLOGIA

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CONTENTS

Radiation mutants of Aspergillus niger.....SIGMUND BERK 723

Acidic metabolic products of Hansenula anomala
STUART B. HUGHES 736

Pathology, morphology and nuclear cycle of two new species of Pyrenophora. MARVIN D. WHITEHEAD AND J. D. DICKSON 747

Phycomycetes from the Douglas Lake region of northern Michigan.....F. K. SPARROW 759

Synonymy of Tilletia horrida and Neovossia barclayana
E. C. TULLIS AND A. G. JOHNSON 773

An undescribed species of ergot on Panicum maximum Jacq. var. Common Guinea.....T. THRIS 789

Western Fungi—III
WM. BRIDGE COOKE AND CHARLES GARDNER SHAW 795

A new species of Heterosporium from soil....R. G. ATKINSON 813

A new species of Fomes from Pakistan..S. MURTAZA HUSSAIN 823

Species of Synchytrium in Louisiana. VII. A new species on Urtica chamaedryoides.....MELVILLE T. COOK 827

Notes and Brief Articles..... 829

Reviews..... 835

Directory..... 838

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MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLIV NOVEMBER–DECEMBER, 1952 No. 6

RADIATION MUTANTS OF ASPERGILLUS NIGER[1]

SIGMUND BERK

(WITH 4 FIGURES)

The work done up to 1935 on the biological effects of ionizing radiation on microorganisms is summarized by Duggar (**6**). More recent and pertinent articles will be reviewed here.

It is now well established that radioactive emanations will produce lethal effects on fungi if the dose is sufficiently great. With sublethal exposures various degrees of injury are produced. Kresling and Stern (**12**) found that mild doses of radiation from radium stimulated citric acid formation in *Aspergillus niger*. Muller (**15**) states that 99.9 per cent of the mutations are detrimental. Sartory *et al.* (**22**) reported that the irradiation of *Aspergillus niger* with a 50 mc. radium source produced the sexual stage of the fungus. Nadson (**17**) investigated the effect of radium on fungi and obtained structural abnormalities that were inherited in successive generations of the organism.

Nakazawa and Simo (**16**) irradiated *Aspergillus niger* with 3.7 mg. of radium and obtained a number of morphological variants.

[1] Extract presented at the Meeting of the Society for Industrial Microbiology held in conjunction with the American Association for the Advancement of Science, Philadelphia, Pa., December 27, 1951. Appreciation is expressed to the Ordnance Corps, Department of the Army for permission to publish this article and to Dr. K. B. Raper for his helpful review of the paper.

[MYCOLOGIA for September–October (**44**: 587–721) was issued October 11, 1952]

Kresling and Stern (**13**) irradiated *Aspergillus niger* on agar culture medium and also observed inhibition of growth and the production of morphological changes in the organism.

Steinberg and Thom (**24**) obtained mutants of *Aspergillus niger* by the action of nitrous acid but when these mutants were grown on certain amino acids he obtained reversions to the parent or original strain. Nitrogen mustard gases are known to be mutagenic agents but the work of Owen *et al.* (**18**) showed that variants of species of *Botrytis* obtained by exposure to the nitrogen mustard gas (methyl-bis (β-chloroethyl) amine) were not stable through repeated transfers.

Raper *et al.* (**21**) described a large number of ultraviolet-induced mutations of *Aspergillus terreus*.

Pearson *et al.* (**19**) exposed *Aspergillus* niger spores for 48 hours to 20 μc. sources of radiophosphorus, P^{32} (a pure 1.7 Mev beta emitter) and radioiodine I^{131} (a beta- and gamma-emitter). No gross morphological changes were observed in the fungus. A decrease in the growth rate was obtained in the spores exposed to the I^{131} and no effect with the P^{32}.

Spores of the normal strains of *Neurospora* were exposed to the atomic bomb radiation at Bikini and Einiwetok (**2**). Many of the mutations found were similar to those produced in the laboratory with X-rays and ultraviolet light. It has been observed that the type of mutation obtained is independent of the mutagenic agent used, e.g., mustard gases, ultraviolet, etc.

In the course of an investigation to determine the effectiveness of ionizing radiation on certain fungi (**3**) a number of radiation mutants of *Aspergillus niger* were obtained and are described in this report.

ASPERGILLUS NIGER FA #300

A single spore isolate from a culture of *Aspergillus niger* van Tiegh. USDA TC *215–4247* was used. This single spore isolate was designated as FA *#300*. Steinberg (**23**) about 1917 isolated the original strain as a single spore culture and designated it as the "W" strain. The "W" strain is differentiated from his (**23**) "Y" strain by the absence of yellow pigment in the hyphae and by the formation of a smaller amount of tissue. The asexual

spores (conidia) are black when ripe, 3.5–4 μ in diameter, smooth at first, later rough. The conidiophores (stalks bearing the spore head apparatus) are 600 to 700 μ long.

All stock cultures of FA *#300* were grown on Difco potato-dextrose agar. In all experimental work, unless stated otherwise, the age of the inoculum used varied from 3 to 6 days. All cultures were incubated in a constant temperature room maintained at $29 \pm 1°$ C.

RADIATION MUTANTS

Although many atypical cultures of *Aspergillus niger* were observed after irradiation with polonium and radium, only a few attempts were made to isolate the variants. There is some difference of opinion on what constitutes a mutation or variant. The term mutation is usually defined as a sudden permanent change in the character of an organism.

In one experiment a modified Czapek's medium was diluted with equal parts of distilled water and the agar content reduced to 0.4 per cent. A drop of spore suspension containing spores of *Aspergillus niger* FA *#300* was added to this medium in culture tubes and agitated to disperse the spores uniformly. A drop of the resulting suspension was placed on glass microscope slides. The radioactive materials were placed at approximately 2, 7, and 25 mm. above the spore drop. The strengths of the radium sources used were 0.28 μg. and 3.7 μg., and of the polonium source, 300 μc. The radium-activated foil and the polonium plated on nickel foils used are described elsewhere (3). The inoculated slides with the radioactive sources were incubated over water for seven days in 200 mm. desiccators.

At the end of the incubation period isolations were made on Czapek's and on potato-dextrose agar. Mutations were detected by examination of the cultures and noting any changes in the cultural and morphological characteristics of the fungus. Some of the cultures were characterized by dwarf and giant conidial heads, by extensive aerial mycelium growth and by sparse or powdery type of conidial growth. To determine whether permanent variants had occurred, subcultures were made from the isolates. It

was found that many of the subcultures yielded variants by sectoring.

One isolate characterized by yellow mycelial growth and sparse sporulation (FA *#301*) was isolated from a Petri dish culture of FA *#300* on potato-dextrose agar which was irradiated with a 3.75

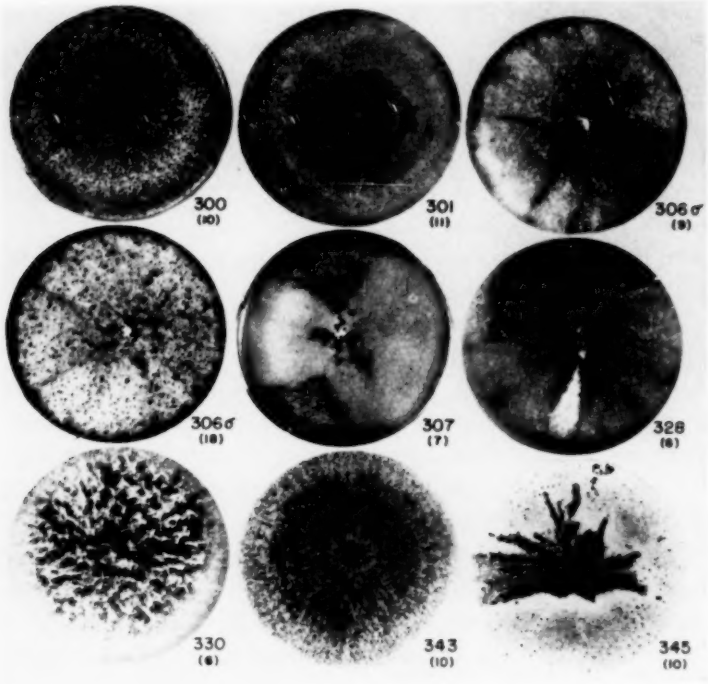

FIG. 1. Comparison of cultural and morphological characters of mutants of *Aspergillus niger* obtained by the irradiation of the parent strain FA *#300* with alpha particles. The numbers in parentheses refer to the number of days the cultures were incubated on potato-dextrose agar.

μg. source of radium for seven days. The isolate was made from the central zone of inhibition of sporulation which is produced by the continuous irradiation of the culture (3).

Ten to 50 subcultures were made from these variants over a period of 2½ years. The original strain of *Aspergillus niger*

showed no variation in six years of subculturing, although most fungi are considered as very variable and mutable. However, after irradiation, the formerly stable isolate developed a marked instability. With the exception of the yellow mycelium mutant type (FA *#301*), successive subcultures of the other mutants in many cases gave variants with sectors different from the original variant.

Since gross transfer of inoculum from the mutants yielded cultures with the characteristic growth inconstant, single spore isolates were made. Subcultures made from the single spore isolates still yielded cultures characterized by sectoring. It appears that the ionizing radiation produced an instability in the "rayed" fungus. Ford (**8**) reported a similar instability in *Chaetomium globosum* irradiated with short wave lengths of ultraviolet. FIG. 1 shows the type of variants obtained when grown on potato-dextrose agar. The sectoring phenomenon is shown in FIG 1 (FA *#307*, *#328*, and *#345*). The mutant cells appear as a wedge-shaped sector in the colony with the apex at the point of the original mutated cell. Dickson (**5**) who worked with the effects of X-rays on a species of *Chaetomium*, also obtained sectors with fruiting bodies of reduced size and some with large amounts of aerial mycelium.

In some cases, variants developed normal colonies on subculturing. Most of the subcultures from such "normals" resulted in normal colonies. However, in a few cases there was a shift from a "normal" back to a mutant. In mutations of *Aspergillus niger*, according to Steinberg (**25**), heterokaryosis is probably not involved. It is believed that a single spore of *Aspergillus niger* contains only a single haploid nucleus. If heterokaryosis were a factor, the reversion from normal to mutants could be explained. Gossop *et al.* (**9**) described the production of "heterocaryotic" heads bearing two types of conidia in mixed cultures of strains of *Aspergillus niger*.

The type of mutants obtained may be characterized as follows: Type I: FA *#301*, when grown on potato-dextrose agar, is stable, yellow-colored, and has a leathery mycelial mat; center of culture may become gelatinous; sporulation is sparse and delayed. When

grown on Czapek's solution agar the fungus is stable, has a yellow to orange leathery mycelium with scant and delayed sporulation.

Type II: In strains FA *#306*, *#307*, *#312*, and *#333A*, when grown on potato-dextrose and Czapek's solution agar, the mycelium is similar to the parent strain with dwarf conidial heads containing many sterigmata with none or a reduced number of conidia. Among the dwarf heads there are occasional outcroppings of giant heads (Fig. 1, FA *#307*). The dwarf heads were about 1/6 the size of the normal heads of the parent and the giant heads were about 3 to 4 times as large as those from the parent strain (FA *#300*).

Type III: In strains FA *#308* and *#345*, when grown on potato-dextrose and Czapek's solution agar, the conidial heads are short stalked and exhibit a powdery type of sporulation.

Type IV: In strain FA *#330*, when grown on potato-dextrose agar, the mycelium shows an aerial type of growth.

Occasionally a variant would show a white or yellow mycelium outcropping (Fig. 1, FA *#328*).

Many of the small conidial head isolates reverted to the normal while practically all the isolates from the giant heads reverted to normal. The short-stalked isolate gave sectors with dwarf heads. Czapek's solution agar was more effective in bringing out variant sectors than potato-dextrose agar.

The variants obtained were observed only for cultural and morphological differences. A great many of the mutants obtained were probably biochemical mutants. The abnormalities of the biochemical mutants would have appeared more readily on Czapek's solution agar, which does not contain amino acids. Hollaender *et al.* (11) state that Czapek's solution agar is an incomplete medium, and that fungi which have lost their ability to synthesize essential compounds by irradiation grow slowly or fail to grow in this medium. On the other hand, potato-dextrose and malt agar are considered more complete media, and the biochemical mutant fungi should grow normally on these media.

A number of mutant strains of *A. niger* obtained by other investigators were compared with those obtained in this study. Two strains (radium strain 1/1 and 3/1 Vitamineninstitut, Leningrad)

of *A. niger* V. Tieghem were received from Dr. J. Westerdijk of the Centraalbureau voor Schimmelcultures of Baarn, Holland. The two strains did not resemble any of the mutants reported here in type of vegetative growth or sporulation.

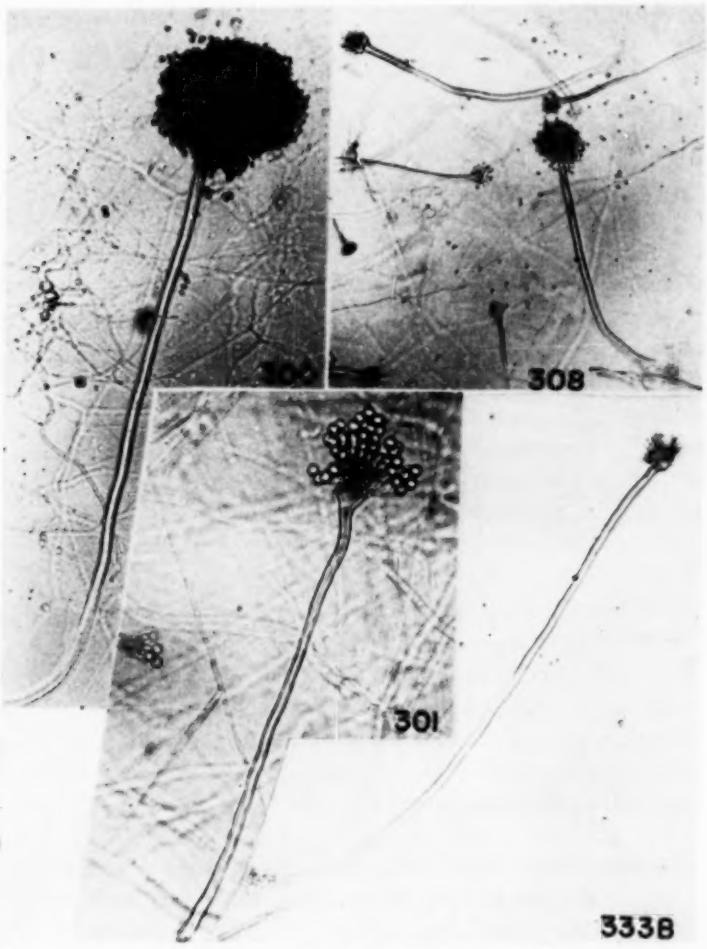

FIG. 2. Comparison of morphological characters of the conidiophores and conidial heads of *Aspergillus niger* (radiation induced) mutants. FA #*300* is the parent strain. All at × 145 except FA #*301* which was photographed at × 295.

Haskins (**10**) parent strain of *Aspergillus niger* (*36.1.1*)[2] resembled the radium mutants that had dwarf conidial heads. The cathode ray induced mutant (C. Haskins, mutant #*2*, *36.1.3*) produced a yellow mycelial pad with sparse sporulation on potato-dextrose agar and was similar to the radium induced mutant FA #*301*. Haskin's mutant #1 (*36.1.2*) had different cultural characteristics than the ionizing radiation induced mutants described in this study.

MICROSCOPIC DIFFERENCES BETWEEN CONIDIAL HEADS OF IONIZING RADIATION INDUCED MUTANTS OF ASPERGILLUS NIGER

The alpha particle radiation induced mutants of *Aspergillus niger* were grown on glass microscope slides on potato-dextrose agar diluted with an equal part of distilled water. After 67 hours' incubation, the slide cultures were fixed and stained with Amann's lactophenol with cotton blue. Culture FA #*301* was fixed after 91 hours' incubation, since no sporulation occurred after 67 hours. The photomicrographs in FIG. 2 show differences in length of conidiophores and size of the conidial heads of the mutants. Cultures FA #*308* and #*301* have short stalks as compared to the parent strain FA #*300*. Culture FA #*333B* has a normal size conidiophore, but the sterigmata bear a reduced number of conidia.

The photomicrographs in FIG. 3 compare the morphological character of the vesicles, sterigmata, and conidia of the mutants after 91 to 120 hours' incubation on diluted potato-dextrose agar. Culture FA #*306* shows a giant head after six weeks' incubation on potato-dextrose agar in a Petri dish. The extreme size of the giant head is due to the large size of the sterigmata and the increased number of conidia present. The alpha particle radiation induced mutants, FA #*301*, #*308*, and #*333B*, had conidial heads bearing fewer conidia than the parent strain FA #*300*.

CLASSIFICATION OF ASPERGILLUS NIGER MUTANTS BY ELECTRON MICROGRAPH DIFFERENCES IN CONIDIA

It was believed that the use of the electron microscope could serve as a means of separating mutants of *Aspergillus niger* on the

[2] Furnished by Dr. C. B. van Niel, Hopkins Marine Station, Pacific Grove, California.

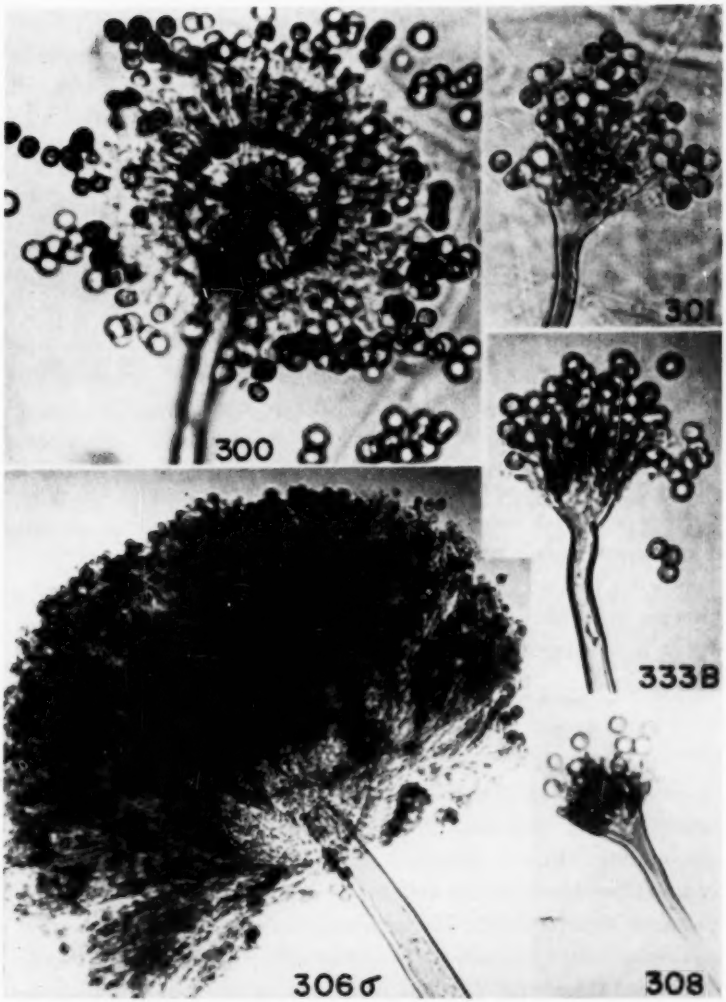

FIG. 3. Comparison of conidial heads with vesicles and sterigmata of radiation mutants of *Aspergillus niger* grown on potato-dextrose agar. The cultures were incubated as follows: FA #*300*—96 hours (parent strain), FA #*301*—91 hours, FA #*333B*—120 hours, FA #*308*—120 hours, and FA #*306σ*—six weeks. All at × 650 except #*306σ* which is × 320.

basis of differences in the morphology of the conidia. FIG. 4 shows electron micrographs of the conidia of the parent strain FA #300 and of three variants. No significant morphological differences in the conidia were observed, except differences in the size of some of the conidia of the variants. It was learned that Favre *et al.* (**7**)

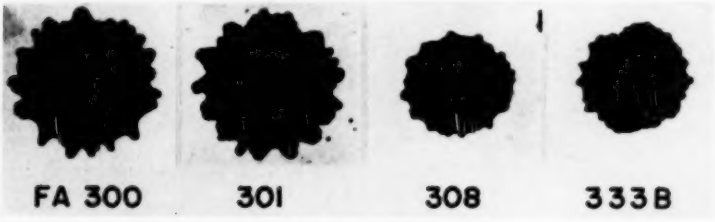

FIG. 4. Electron micrographs of conidia of *Aspergillus niger* mutants. FA #300 is the parent strain. × 4700.

also suggested the use of the electron microscope, which has a resolving power 100 times greater than that of the optical microscope, for the taxonomic description of fungus spores. Randall (**20**) points out that no gross shrinkages result from the vacuum drying process to which electron microscope specimens are subjected, but there is a danger in artifact production since specimens are examined in vacuo.

DISCUSSION

That ionizing radiation is responsible for many biological effects is well known. Large doses of high energy radiation lead to the death of cells. Sublethal doses produce abnormalities, variations, and mutations in the genetic makeup of the cell or nucleus. The changes produced in the cell may be temporary (reversible) or permanent (irreversible) depending on the dosage.

Catcheside (**4**) classifies genetically effective radiations into two types: (1) ionizing (alpha-, beta-, and gamma-radiations from radioactive substances, X-rays, protons, and neutrons) and (2) nonionizing (ultraviolet light). The ionizing type produces both ionization and excitation of molecules while the nonionizing type produces only excitation of molecules.

Ionizing radiations cause mutations at a rate in excess of the spontaneous rate. Lea (**14**) states that a dose of the order of 10^7 to 10^8 roentgen is required for an average of one mutation per gene.

Beadle (**2**) gives three methods by which high energy radiation may produce genetic changes in cells. The first method entails the formation of toxic materials and free radicals in the cell. The second involves the direct ionization of organic molecules which results in the breakage of chemical bonds. The third produces chromosome breaks.

Catchside (**4**) divides mutations obtained by radiations into two types: (1) morphological or cultural, which are detected by alterations in color, appearance, size or rate of growth of the fungus, and (2) losses in biosynthetic capacity, which are detected by failure of mutant isolates to grow on the basal or minimal medium (i.e., the medium on which the normal or parent shows good growth).

Stone *et al.* (**26**) irradiated culture medium with 2537A° UV and found that the irradiation of the amino acids and vitamins resulted in a marked increase in mutation rate. They also found that a good portion of the toxic effect arose from the irradiated glucose. The mutations produced in this experiment may be attributed not only to the ionization of the fungus tissue by the alpha particles but also, as shown by Stone *et al.* (**26**), to the activation of the chemicals in the culture medium. The activated chemicals, "mutators," when utilized by the growing mycelium may cause mutations in the fungus.

Ionizing radiations are injurious to cells that absorb them. Gray (**27**) reported that the radiation interferes with almost every known function of the cell when delivered in sufficient dosage. There is increasing evidence that ionizing radiation destroys enzymes and interferes with the carbohydrate metabolism of the cell.

Barron and Dickman (**1**) reported that ionizing radiations have two different actions on proteins: (1) the oxidation of $-$ SH groups which is a reversible phenomenon, and (2) the denaturation and destruction of the molecule which is an irreversible phenomenon. It is known that an abundance of sulfhydryl compounds are required by cells in cell division and growth.

SUMMARY

Ionizing radiations from radium and polonium were used to produce a number of mutants in a monosporous strain of *Aspergillus niger*. The alpha radiation is considered responsible for the mutant strains obtained. A yellow-colored mycelial strain FA #301 was found to be stable. The other variants had varying degrees of instability and tendencies to form sectors. Some of the variants were characterized by dwarf and giant conidial heads. Morphological differences in the mutants were determined by microscopic examination.

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ACIDIC METABOLIC PRODUCTS OF HANSENULA ANOMALA[1]

STUART B. HUGHES

(WITH 3 FIGURES)

The volatile neutral metabolic products identified in medium fermented by *Hansenula anomala* (Hansen) Sydow[2] are acetaldehyde, ethyl alcohol, and ethyl acetate. These substances were isolated and identified by Gray (**1949**) and reaffirmed by Davies *et al.* (**1951**). Davies also identified acetic acid by chromatographic means and detected a non-volatile acid which did not give positive qualitative tests for succinic, lactic, or pyruvic acids. Tabachnick (**1951**) by means of paper chromatography detected small amounts of malic, succinic, citric and fumaric acid, besides acetic acid in the yeast cells.

This work was undertaken for the purpose of identifying if possible any acidic products synthesized by this yeast in the fermentation medium.

MATERIALS AND METHODS

The basal medium used by Kuehner (**1951**) in his studies on the effect of vitamins and micrometabolic elements on growth and ethyl acetate production by *H. anomala* was adopted for use in this problem since it was a chemically definable medium.

In preparing inoculum for use in these studies a 100 ml. portion of basal medium (TABLE I) was autoclaved at 15 pounds gauge pressure for 20 minutes, cooled, and inoculated with a loop needle from a three-day-old agar slant of *H. anomala* (cultured on the following medium: 0.7 per cent Difco Yeast Extract, 0.5 per cent

[1] The Ohio State University, Department of Botany and Plant Pathology. Publication No. 540.

[2] The strain investigated is known to be the same as that used by Gray and Kuehner. It was obtained originally in 1939 from the Dept. of Ag. Bact., University of Wisconsin.

KH_2PO_4, 5.0 per cent Dextrose, and 3.0 per cent agar agar). This liquid culture was then incubated for 24 hours at 30 ± 2° C. At the end of the incubation period the yeast from this 100 ml. culture was collected in a sterile 50 ml. centrifuge tube. The yeast cells were washed once with sterile M/15 KH_2PO_4, recentrifuged, and then resuspended in 20 ml. of this solution. Potassium phosphate solution was used in these preliminary steps to maintain the viability of the yeast cells. Immediately following the final resuspension of the concentrated cells, counts were made using a Spencer Bright Line Haemocytometer. The number of milliliters of this suspension necessary to provide the desired number of yeast cells was calcu-

TABLE I

COMPOSITION OF BASAL MEDIUM

Constituent		Per liter of solution
Dextrose	C. P.	20.0 grams
Asparagine	C. P.	2.0 grams
KH_2PO_4	C. P.	1.5 grams
$MgSO_4 \cdot 7H_2O$	C. P.	0.5 grams
$CaCl_2$ (Anhyd.)	C. P.	0.28 grams
$(NH_4)_2SO_4$	C. P.	2.0 grams
KI	C. P.	0.1 milligrams
*Trace element solution		1.0 ml.

* Composition of trace element solution.

Element	p.p.m./ml.
$Mn(MnSO_4)$	0.01
$Zn(ZnCl_2)$	0.07
$B(H_2BO_4)$	0.01
$Cu(CuCl_2)$	0.01
$Mo(Mo_2O_3)$	0.01
$Fe(FeSO_4)$	0.05

lated on the basis of these cell counts. The errors incurred in this method were found, by averaging several counts, to be of the order of plus or minus two million when concentrations of about 40 million cells were counted. Sterile 100 ml. portions of basal medium were inoculated with portions of this cell suspension with sterile, graduated, 1 ml. pipettes.

In determining the level of acid production at any specified time during fermentation, a 20 ml. aliquot was taken from one flask culture of a series of nine 100 ml. cultures. These 20 ml. aliquots were titrated with 0.1008 *N* NaOH using phenolphthalein as indicator. The results of these titrations are reported as ml. of standard

base per 100 ml. of fermentation medium in TABLES III and IV and as standard base per 20 ml. and 5 ml. of medium in FIGS. 2 and 3, respectively.

The information obtained from these trial runs established the optimal inoculum size and incubation period for the maximum production of acid.

One fermentation series was set up to determine what portion of the acids formed in the fermentation of this yeast were volatile. This series was prepared in the manner described above. The volatile acid fraction was determined in the following manner: 5 ml. of the fermentation medium and three drops of syrupy phosphoric acid were put in a Sellier tube in a modified Hortvet apparatus and 100 ml. of steam distillate was collected. This distillate was then titrated with 0.1008 *N* NaOH against phenolphthalein as indicator. This technique was tested on known solutions of acetic, propionic, and butyric acid. The recovery of these volatile acids by this means fell within a range of 99.0 to 100.1 per cent. The total acidity was determined in this series by titrating 5 ml. aliquots of the fermented medium rather than 20 ml. aliquots of the fermented medium as was done in all other determinations of total acidity.

In order to obtain a large sample of the fermentation acids for analysis, thirty 100 ml. portions of basal medium, to which no calcium chloride was added, were sterilized and inoculated with 40 million cells each. The observed effect of added calcium on this fermentation is discussed in the section Results and Discussion. These cultures were then incubated for nine days at 30 ± 2° C. Following this incubation period the medium from all thirty liquid cultures was combined and the yeast cells were centrifuged out of the medium. The supernatant, to which was added one gram of $HgCl_2$ per liter was placed in a refrigerator until it could be analyzed.

The fermented medium was continuously extracted with ethyl ether, 1.5 liters at one time, for four days. The combined ether extracts were then neutralized to phenolphthalein with K_2CO_3. The solution containing the potassium salts was then extracted with ether to remove neutral ether soluble compounds. These salts were then acidified with syrupy phosphoric acid to regenerate the free acids. These acids were then extracted from this acid solu-

tion with ether. The ether was removed by distillation at reduced pressure (about 10 mm. Hg) at a temperature of 35–37° C. A portion of absolute ether equal to that removed was added and distilled off under the same conditions to further dry the acid mixture. These acids were then separated on a silicic acid adsorption column, following the standard procedure outlined by Marvel and Rands (**1950**).

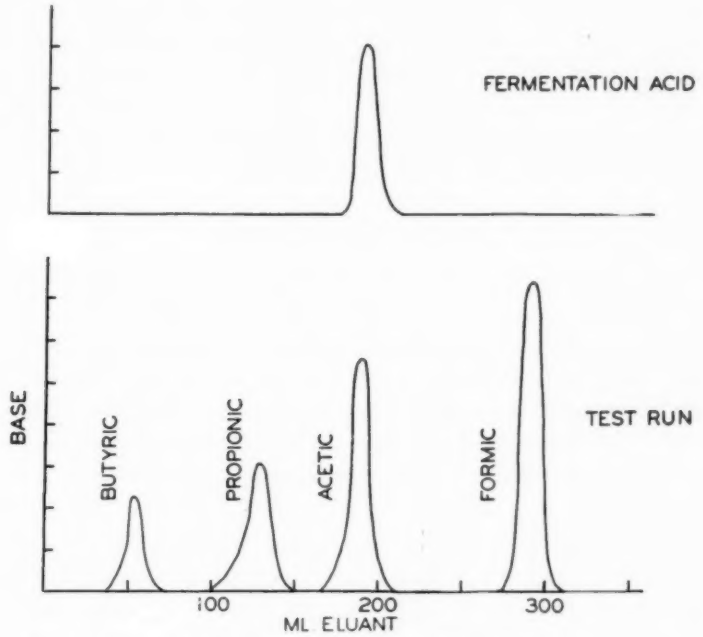

FIG. 1. Results of fractionation of known and unknown acid mixtures on a silicic acid adsorption column following the standard procedure of Marvel and Rands.

Before attempting to separate the unknown acids on the silicic acid adsorption column, mixtures of several common volatile acids (formic, acetic, propionic, and butyric) were fractionated on the column. The values for the peak effluent volumes of these acids agreed closely with the values recorded by Marvel and Rands for the same acids. The results of one trial run are given in the lower half of FIG. 1 and another in TABLE II. The low recovery of

TABLE II

RECOVERY OF VOLATILE ACIDS FRACTIONED ON THE SILICIC ACID ADSORPTION COLUMN

Acid	Added to column (milliequivalents)	Recovered (milliequivalents)
Butyric	0.1989	0.1990
Propionic	0.2392	0.2460
Acetic	0.2117	0.2140
Formic	0.4109	0.3620

formic acid shown in TABLE II is due to the fact that the elution was stopped prior to the collection of the eluant containing the last traces of the formic acid fraction. The recovery, however, was sufficient to permit identification of this fraction as formic acid. The results of these trial runs were reproducible and agreed closely with the data reported by Marvel and Rands.

RESULTS AND DISCUSSION

The relation of inoculum size and length of the incubation period to the production of acidity is shown in FIG. 2. The results indicate that the smaller inocula (14 and 40 million cells per 100 ml. basal medium) resulted in the production of a greater amount of acid. In contrast to this, Gray and Barton (unpublished data) found that the optimal inoculum size for maximum ethyl acetate production was about 76 million cells per 100 ml. of basal medium. Considerable variability in acid production was noted. The total acid determinations on 11 replicate flasks taken from three separate series are presented in TABLE III. Each of these cultures was inoculated with 40 million cells and incubated for 13 days at 30 ±

TABLE III

VARIABILITY OF ACID PRODUCTION BY *Hansenula anomala*

Series number	ml. 0.1008 *N* NaOH per 100 ml. medium			
	Flask numbers			
	6	7	8	9
9	34.0	31.0	30.5	20.0
10		24.0	22.4	19.5
11	29.0	26.5	23.0	19.0

2° C. The data in TABLE III were calculated from results obtained by titrating 20 ml. aliquots of fermented medium with 0.1008 *N* NaOH (against phenolphthalein).

Data on the production of volatile and total acidity in one fermentation series is presented in TABLE IV. The values recorded in TABLE IV were calculated from titrations of the acids contained in 5 ml. aliquots of fermented medium (volatile acids by titration of 100 ml. of steam distillate; total acidity by direct titration of 5 ml. aliquots).

As shown above, the volatile acidic fraction of the fermentation acids composed 90 per cent of the total acidity after twelve days and fell to 50 per cent on the fourteenth day. These flasks were all checked for bacterial contamination and none was found. The

TABLE IV

TOTAL AND VOLATILE ACIDITY PRODUCED IN FERMENTATION MEDIUM BY *Hansenula anomala*

Forty million cell inoculum (expressed as ml. 0.1008 *N* NaOH per 100 ml. medium)

Incubation period (days)	8	9	11	12	14
Total acid	36.0	34.0	44.0	40.0	48.0
Volatile acid	16.0	14.0	22.0	36.0	24.0

high total acidity present on the fourteenth day is in marked contrast to the usual fermentation in which the acids show marked decrease in concentration on the tenth or eleventh day.

It was thought that the presence of calcium chloride in the basal medium might precipitate certain acids as insoluble calcium salts which are not soluble in ether, and thus obscure their presence. The effect of calcium on the fermentation was investigated to ascertain the advisability of excluding calcium chloride from the medium. When calcium was not included in the basal medium no appreciable difference in appearance and disappearance of total acidity as observed in other fermentations was noted. As shown in FIG. 3 the total and volatile acidity reached a maximum after ten days of incubation. This compares with the acid curve in FIG. 2 in which 40 million cells per 100 ml. medium was used as inoculum. The same

size inoculum was used in the fermentation series which contained no added calcium.

The presence of calcium contaminants in analytical grade chemicals is reported by Richards and Troutman (**1940**). Since no attempt was made to remove the contaminating calcium from the chemical constituents of the basal medium no conclusion can be drawn as to the calcium requirement of this yeast. However, it was noted in two comparable cases that the cell count in an inoculum flask (incubated for one day) which contained added cal-

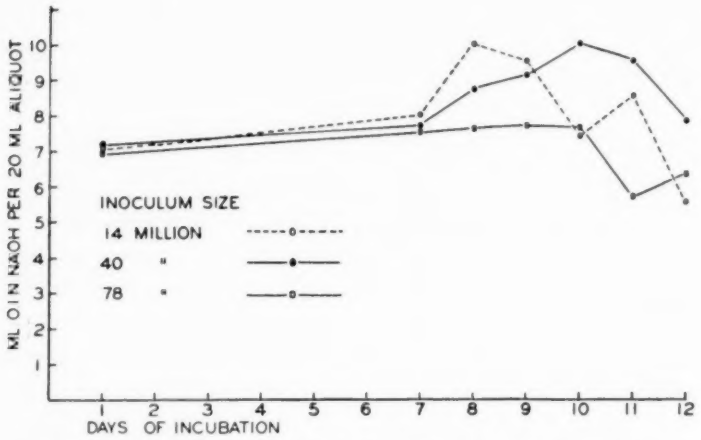

FIG. 2. Acidity produced in the fermentation of basal medium by *Hansenula anomala*.

cium chloride was 30 per cent greater than that of a similar inoculum flask which contained no added calcium chloride.

The acids present in the fermentation medium were removed from the medium by ether extraction and a separation of the acid constituents was attempted using the technique of Marvel and Rands (**1950**). Acetic acid was tentatively identified in this manner, no other volatile acid being detected. A para-bromophenacylbromide derivative of this supposed acetic acid fraction was prepared following the procedure outlined by McElvain (**1947**), and found to melt at 85°–86° C. (uncorrected). McElvain gives the melting point of para-bromophenacyl acetate as 85° C. The titra-

tion curve showing the characteristic acetic acid peak obtained from the unknown fermentation acids (by fractionation on the silicic acid adsorption column) is compared with the curve obtained with known acids in FIG. 1.

In the course of the adsorption column studies it was noted that a considerable portion of the unknown acid mixture added to the column was not detected in the eluant when the process of elution was continued through 1100 ml. of the eluting solutions. This

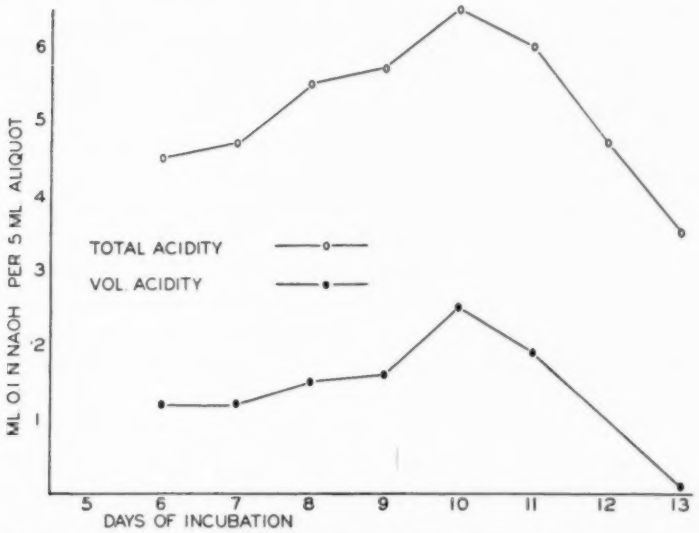

FIG. 3. Production of total and volatile acid in medium containing no added $CaCl_2$ by *Hansenula anomala*.

undetected acid fraction was soluble in *n*-butanol, ether, and water but not soluble in chloroform. An attempt was made to purify this acid in two ways: (1) the acid was dissolved in ether and precipitated out of the ether solution with chloroform followed by washing on a sintered glass filter (with chloroform), (2) the acid was dissolved in a minimum of *n*-butanol and this solution was diluted with 20 times its volume of chloroform. This solution was allowed to stand overnight at room temperature whereupon a crystalline substance formed on the bottom of the flask.

The acidic material obtained by the first procedure was water soluble and had a neutralization equivalent of 155 (0.0487 g. was equivalent to 3.13 ml. 0.1008 *N* NaOH). This acid was titrated potentiometrically and there was no indication of more than one acidic group being present. The para-bromophenacyl ester of this acid melted at 181°–182° C. (uncorrected). This acid did not decolorize bromine-carbon tetrachloride solution and decomposed without melting at 212°–217° C. (uncorrected). The available tables of derivatives did not list any acids with the above characteristics.

The material which was obtained by the second method was obviously different from the original acid, since it was insoluble in water and not appreciably soluble in ether or *n*-butanol. This solid material was titrated in water, a neutralization equivalent of 217 being obtained. If this compound was an easily saponified esterification product of the acid and *n*-butanol one would expect the neutralization equivalent to be about 229. This value was reached by adding the molecular weight of *n*-butanol (74) to the neutralization equivalent of the water soluble acidic compound (155). The two values, 217 and 229, are close enough to indicate that an esterification reaction probably occurred between the acid and the alcohol. If this reaction does occur it would have done so on the silicic acid adsorption column and would explain the inability to detect this acid by this means. Using the standard procedure of Marvel and Rands (**1950**), this acid was in contact with *n*-butanol for ample time to permit the esterification reaction to take place (19 hours being required to pass 1100 ml. of the effluent mixtures through the silicic acid column). If this reaction did take place, as is strongly indicated, neither the free acid (as added to the column) nor the butyl ester of this acid (formed on the column) would have been detected in the eluant, since the former would not have existed in detectable amounts and the latter would have been insoluble in the chloroform-*n*-butanol effluent mixtures used. Further development of this adsorption technique with respect to the repression of esterification reactions on the column would provide the research worker with an extremely useful tool for the analysis of complex fermentation acid mixtures.

SUMMARY

1. Optimal inoculum size and length of incubation period necessary for the maximum production of acidic metabolic products in medium fermented by the yeast *Hansenula anomala* (Hansen) Sydow was determined.

2. The inclusion of calcium chloride was shown to have no significant effect on the production of fermentation acidity by this yeast, but was found to have an accelerating effect on cell multiplication, at least during the first day of incubation.

3. Only two acids were detected in the medium fermented by this yeast; a volatile acid (proven to be acetic), and a non-volatile acid which was not identified. This acid decomposed (without melting) at 212°–217° C. It was shown to be saturated and to contain only one acidic group. The neutralization equivalent was 155 and the para-bromophenacyl ester melted at 181°–182° C. Some evidence indicating that this acid readily forms an esterification product with *n*-butanol is presented.

4. The silicic acid adsorption column technique for the separation and identification of components of unknown acid mixtures as described by Marvel and Rands was found to be satisfactory for the purification and identification of four common volatile acids (formic, acetic, propionic, and butyric) but not for an acid which is easily esterified with *n*-butanol.

I wish to express appreciation to Dr. William D. Gray for his service in an advisory capacity throughout the course of this work.

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PATHOLOGY, MORPHOLOGY AND NUCLEAR CYCLE OF TWO NEW SPECIES OF PYRENOPHORA [1]

MARVIN D. WHITEHEAD AND J. G. DICKSON

(WITH 29 FIGURES)

Two apparently undescribed species of *Pyrenophora*, overwintering prevalently on oat and rye stubble and straw in Wisconsin, were connected with their imperfect stages and their morphology and cytology described. The *Pyrenophora* sp. on oat straw has seemingly been confused with *Pyrenophora avenae* (Eidam) Ito and Kuribayashi for many years. The species on rye was first noted by the junior author in the fall of 1945.

The material for microscopic study was fixed in Karpechenko's modification of Navashin's fluid. Smears of asci and ascospores on slides were stained with Newton's iodine gentian violet or phosphate-ripened iron hematoxylin and counterstained with alcoholic solution of picric acid or Orange G. Single spore isolates of ascospores and conidia were cultured on potato-cerulose agar and straw-water agar.

PYRENOPHORA ON OAT STRAW

The *Pyrenophora* species commonly occurring on oat straw in Wisconsin during the past two years was found to have an *Alternaria* conidial stage. As far as the authors have been able to determine, it is the first reference of a *Pyrenophora* species, *Pleospora* species according to Wehmeyer (**7**), with an *Alternaria* conidial stage. Stevens (**6**) gives Diedicke (**1, 2**) credit for first observing this phenomenon in connection with the culture of ascospores of

[1] Cooperative investigations between the University of Wisconsin and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture.

Pleospora trichostoma (Fr.) Wint. Translation of the two papers concerned revealed that Diedicke did not state that he had obtained *Alternaria* from the ascospores but that he had obtained *Helminthosporium* and *Alternaria* in mixed culture and that with further platings he had obtained only *Helminthosporium*. Many references have been made connecting the genus *Pleospora* to the imperfect genus *Alternaria*, but Neergaard (**5**), in his monographic treatment of the genus states, "as is evident from the investigations cited, certain *Alternaria* appear to be able to develop a *Pleospora* stage, but sufficiently thorough studies on this question are still wanting, for one thing, in order to establish indisputably which species possess this faculty." At least in all this varied cultural studies, he did not obtain life cycle or morphological evidence that would suggest perithecial formation of *Alternaria tennis* Auct., the species several times connected with *Pleospora*.

It is not known whether the species occurring on oat straw develops parasitically under field conditions. In greenhouse tests a moderately severe killing of leaf tissue was observed when plants were spray-inoculated with mycelial fragments and conidia and left in the moist chamber for 48 hours.

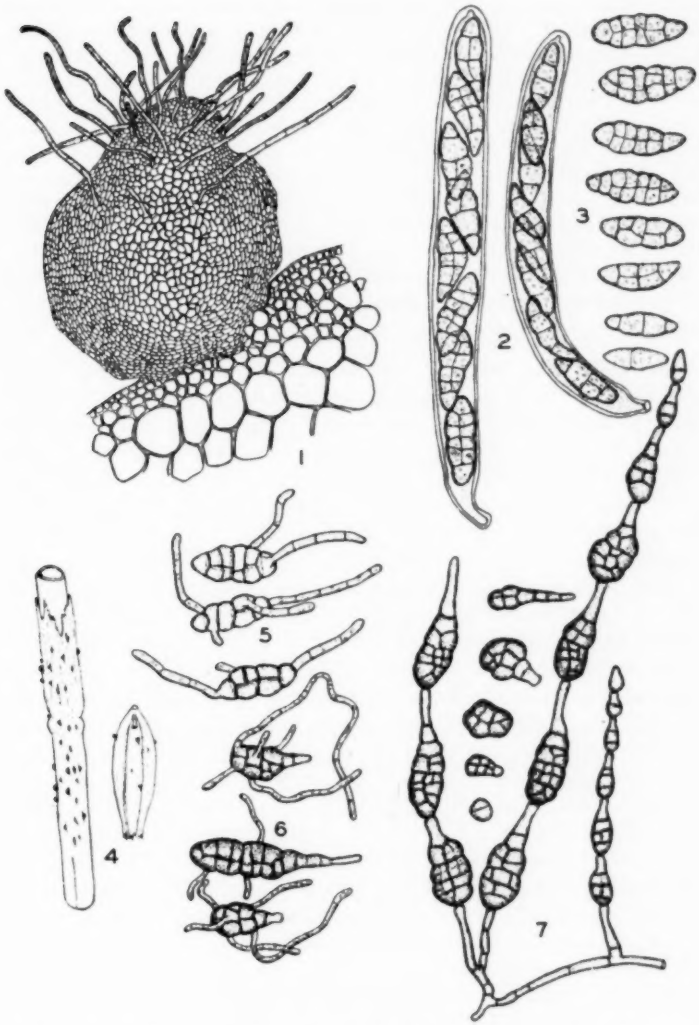
Single spore cultures of ascospores on potato-cerulose agar produced colonies of dark dull gray, moderately profuse aerial mycelium. Conidiophores with chains of conidia of an *Alternaria* (FIG. 7) developed over the larger portion of the colony. A considerable number of sterile, subspherical, dark sclerotia not unlike the perithecial initials formed in nature developed in the mycelium of the cultures.

DIAGNOSIS

Pyrenophora alternarina sp. nov.[2]

Perithecia in graminibus, vaginis, et culmis, primum subepidermica dein erumpentia, dispersa, in sectione horizontali globosa, longitudinali elliptica, ex evolutione rostrorum brevium ostiolarium lageniformia, pariete aureobrunneo, subpseudoparenchymatico, 195–397 μ in diametro, 212–371 μ altae, setis aureo-brunneis, 35–150 μ longis, cylindricis, apicem rotundatum versus subattenuatis, curvatis vel sinuosis, 0–10-septatis praedita; conidiophoris nullis;

[2] The authors are indebted to Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, U.S.D.A., for the preparation of the Latin diagnosis.



FIGS. 1–7. Drawings of habitat and morphological characters of *Pyrenophora alternarina*. 1. Perithecia in short diameter (straw cross section) view, × 440. 2, 3. Asci and ascospores, × 1900. 4. Habitat, oat straw, and kernel showing perithecia, × 5. 5. Germinating ascospores, × 1900. 6. Germinating conidia, × 1320. 7. Mycelium and conidiophores with chains of conidia, × 1320.

asci fasciculati, cylindrici, recti vel subcurvati, ad apicem rotundati et ad basin brevi-pedicellati, 78–134 × 9–14 μ, pariete hyaline, firmo, in juventate 3–6 μ, in maturitate tenuiori 0.5–1.5 μ crasso, 1–8-(vulgo 8-)spori; ascosporae 1–2-seriatae, pallide flavidulae, utrinque rotundatae vel leniter elongatae, transverse 3–6-, longitudinaliter 1–5-septatae, ad septa constriculae, 14.3–24.3 × 5.7–7.6 μ. Status conidicus—*Alternaria:* hyphae vetustae atro-olivaceo-griseae, septatae, 2–4 μ latae, demum ad septa constrictae, ramosae; conidiophora olivaceo-bubalina, septata, 47–78 × 3.38 μ, ramosa, recta, interdum extremitate subinflata; conidia 2–8-catenulata, cylindrica vel obclavata, aureo-olivacea usque bubalina, 15.6–65.6 μ longa, rostro 3.1–18.7 μ longo pallidiori incluso, 9.4–21.8 μ lata, transverse 3–8-, longitudinaliter 1–8-septata.

In culmis marcescentibus et graminibus *Avenae sativae*, Wisconsin.

Occurring saprophytically on stubble, decaying straw and kernels of *Avena sativa* L.

Perithecia developing on kernels, leaf sheaths, nodes, internodes, and straw cavity of the oat plant. At first subepidermal then erumpent scattered globose in cross-section and elliptical in longitudinal section becoming flask-shaped by the formation of short ostiolar beaks; wall dark golden-brown moderately pseudoparenchymatous, provided with a tuft of setae and no conidiophores, perithecia 195–358 μ (mean 261.3) in short diameter, 225–397 μ (mean 318.0) in long diameter, 212–371 μ (mean 288.8) in height; setae dull golden-brown, 35–150 μ long, cylindrical, with only slight taper to the rounded end, curved to crooked, 0–10 septate. Asci fasciculate, cylindrical, straight to slightly curved with rounded apex and short stipitate base, 9–14 × 78–134 μ (mean 11.0 × 111.9) long, wall hyaline, firm 3–6 μ thick when young and becoming thin on maturity, 0.5–1.5 μ, 1–8-spored, primarily 8, ascospores uniseriate becoming biseriate. Ascospores light yellowish, ellipsoidal, rounded at both ends to slightly elongate at one or both ends, with 3–6, mostly 5, transverse septa, 1–5 longitudinal septa, occasionally two longitudinal septa occurring in a spore segment; slightly constricted at the septa; 14.3–24.3 × 5.7–7.6 μ (mean 10.3 × 7.1). Center septa are first formed followed by the formation of a septum in each of the end segments of the spore. Longitudinal septa are then formed in the two center segments followed by formation of transverse septa in the center segments. Mature ascospores and conidia are multinucleate cells. Germ tubes develop from any cell of the spore.

Alternaria sp.

Hyphae dark olive-gray, septate 2–4 μ wide, older hyphae becoming constricted at septa, branching; conidiophores olive-buff, septate, 47–78 μ (mean 56.6) long, averaging 3.38 μ wide, branching, erect, sometimes slightly swollen terminally; conidia forming short

chains of 2–8, smooth to irregular in outline, cylindrical or obclavate, golden-olive to buff, 15.6–65.5 μ (mean 40.4) long including spore beak, 9.4–21.8 μ (mean 14.8) wide, 3–8 (mean 4.66) transverse septa, 1–8 (mean 2.7) longitudinal septa, beak 3.1–18.7 μ (mean 10.9) long, of lighter color.

Single conidial cultures on potato-cerulose agar produced colonies of dark gray, moderately profuse, velvety aerial mycelium with scattered tufts of white.

Habitat: Saprophytic on *Avena sativa* L. stubble and straw collected at Madison, Wisconsin, May 1, 1947 (Type); Marshall, Shannon, Argyle, and Gratiot, Wisconsin, May 7, 1947; Cuba City, Cross Plains, Janesville, and Hillsboro, Wisconsin, May 19, 1947; and Hancock, Johnson Creek, Watertown, Jefferson Junction, and Waterloo, Wisconsin, June 10, 1947.

The fungus differs from *Pyrenophora avenae* on *Avena sativa* by over-all morphological measurements being less than half as large as those of *Pyrenophora avenae* and the presence of the full complement of ascospores.

PYRENOPHORA ON RYE

A relatively severe leaf spot of rye seedlings, noticed in the early spring of 1947, was found to be caused by the ascosporic inoculum of a *Pyrenophora* sp. (FIG. 19A). The same symptoms appeared in the fall of 1947 before the ascospores had matured; investigation showed an abundance of *Helminthosporium* conidia produced on the decaying straw in association with the perithecial initials (FIG. 18). Rye seedlings inoculated with cultures of these conidia showed typical symptoms of the disease. The initial infections resulted in restricted necrotic areas that later spread across the leaf causing apical killing (FIGS. 13 and 19A). Conidiophores and conidia were produced abundantly on these necrotic spots and leaf tips.

Single spore cultures of ascospores and conidia of the pathogen produced colonies of dark gray, loose-growing mycelium topped by scattered white tufts. Typical conidia were noted in culture and perithecial sclerotia developed in two-week-old cultures from both ascospores and conidia. These perithecial-sclerotial bodies attained the size and morphological characteristics of naturally produced perithecia but remained sterile.

DIAGNOSIS

Pyrenophora secalis sp. nov.

Perithecia in vaginis culmisque primum subepidermica dein erumpentia, dispersa, in sectione horizontali globosa, longitudinali elliptica, demum ex evolutione rostrorum brevium ostiolarium lageniformia, pariete aureobrunneo, crasse pseudoparenchymatico, 212–729 μ in diam., 199–464 μ alta, setis abundantibus aureobrunneis, 37–140 μ longis, ad basin 5–10 μ in diam., extremitatem rotundatam versus attenuatis, rectis vel subcurvatis, 0–5-septatis praedita, conidiophoris nullis; asci fasciculati; longe clavati usque cylindrici, subcurvati, apice rotundati et basi brevi-pedicellati, pariete hyalino, firmo, initio 9–15 μ, in maturitate tenuiori, 3–5 μ crasso, 185–398 μ longi, 31–51 μ lati, 1–8 spori; ascosporae biseriatae usque uniseriatae, 34–67 × 15–28 μ, pallide flavidulae, ellipsoideae, utrinque rotundatae, tunica gelatinosa tectae, 3-septatae, loculis mediis duabus interdum 1–2 septis longitudinalibus divisis, ad septa constrictulis. Status conidicus—*Helminthosporium*, in foliis; conidiophora aureobrunnea, 8–12 × 93–795 μ, singularia vel 2–3-caespitosa e cellulis epidermicis vel stomatibus orientia, typice dichotome ramosa, ad apices geniculorum conspicuorum cicatrosa; conidia deinceps orientia, hyalina usque pallide flavidula, 1–9-septata, recta, glabra, crasse tunicata, 31–165 × 9–22 μ, recta vel apice subattenuata, utrinque rotundata vel 1-catenulata, cellula connectenti praesenti vel absenti, conidiophoro apicali et conidiis brevibus secundariis e conidio primario magno interdum orientibus.

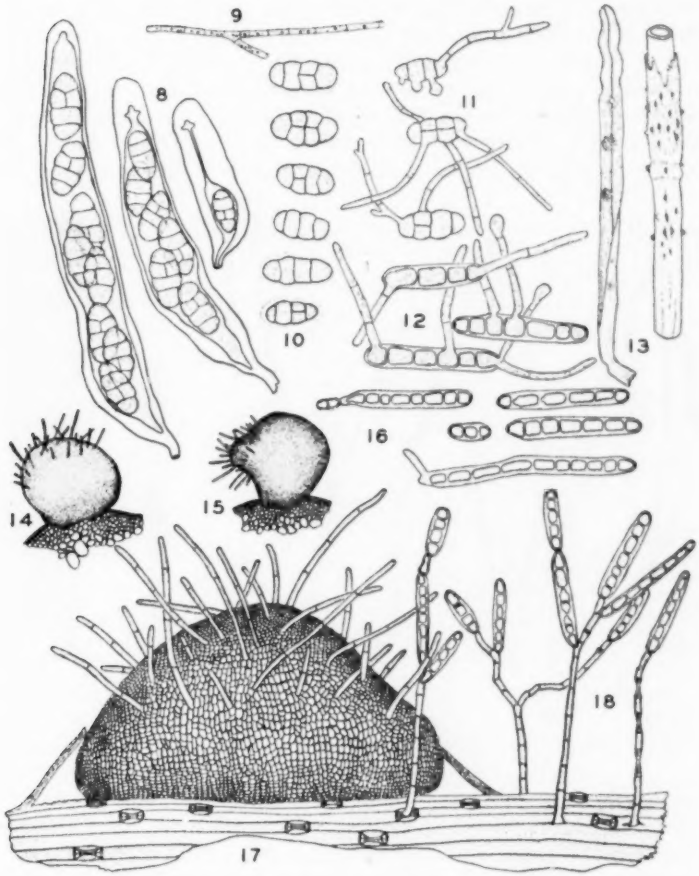
Hab. in culmis marcescentibus foliisque *Secalis cerealis*, Wisconsin.

Occurs saprophytically on stubble and decaying straw of *Secale cereale* L.

Perithecia develop on the leaf sheaths, nodes, internodes, and straw cavity of the host plants that were infected during the period of growth. Perithecia were at first subepidermal, then erumpent, scattered, globose in cross section and elliptical in longitudinal section, becoming flask-shaped by the formation of short ostiolar beaks. The wall is dark golden-brown, heavy pseudoparenchymatous, provided with abundant setae and no conidiophores; perithecia 212–424 μ (mean 318) in short diameter, 424–729 μ (mean 551.7) in long diameter, 199–464 μ (mean 345.9) high; setae golden-brown 37–140 μ long, 5–10 μ at base and tapering to the rounded end, straight or slightly curved, 0–5 septate; asci fasciculate, long-clavate to cylindrical, slightly curved with rounded apex and short stipitate base, wall hyaline, firm, 9–15 μ thick when young and becoming thin, 3–5 μ at maturity, 185–398 × 31–50 μ (mean 267.1 × 41.7) with 1–8, usually less than eight ascospores. Ascospores biseriate becoming uniseriate, 34–67 × 15–28 μ (mean 51 × 21), light yellowish, ellipsoidal, rounded at both ends with outer gelatinous sheath, 3-septate, with or without 1–2 longitudinal septa of the two center cells, slightly constricted at the septum, center septum first formed, followed by end septa then longitudinal septa in the two center segments, germinating from any cell.

Helminthosporium

The foliage of *Secale cereale* L. is infected, producing at first tan restricted spots that later spread across the leaf as a tip necrosis.



FIGS. 8–18. Drawings of habitat and morphological characters of *Pyrenophora secalis*. 8. Mature asci, × 440. 9. Mycelium showing multinucleate condition, × 440. 10. Ascospores, some showing gelatinous sheath, × 440. 11. Germinating ascospores, × 440. 12. Germinating conidia with appressorium-like bodies forming at end of germ tube, × 528. 13. Habitat: leaf showing symptoms, × 2; straw showing perithecial development, × 5. 14, 15. Perithecia in short diameter (straw cross section) view, × 120. 16. Conidia, × 440. 17, 18. Perithecia in long diameter, conidiophores and conidia on straw, × 284.

Conidiophores golden-brown, 93–795 × 8–12 μ, arising singly or in groups of 2–3 usually between epidermal cells, occasionally from stomata, typically branching dichotomously, occasionally alternate cells of the conidiophore showing characteristic shrinkage to approximately one-half diameter, producing the first spore at a distance of 50–260 μ from base and successive spores singly at intervals of 5–40 μ; groups appearing fasiculate, the point of attachment marked by scars at the apices of pronounced geniculations. Conidia hyaline to light yellowish, 1–9-septate (mean 4.5), straight, smooth, thick-walled, 31–165 × 9–22 μ (mean 88.7 × 15.7) measured at widest portion or first or second cell from base, typically straight

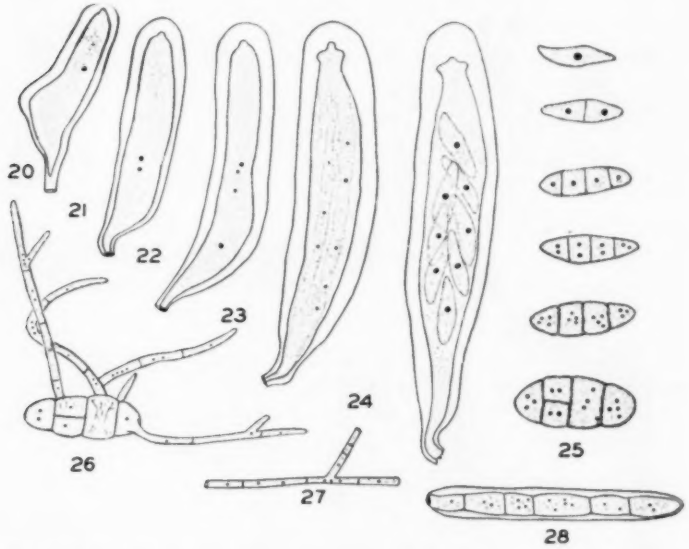
FIG. 19. Photographs showing symptoms caused by *Pyrenophora secalis* on rye. A. Leaf spot and necrosis on rye seedlings. B. Perithecia on rye straw, × 1.5. C. Perithecia, conidia and conidiophores on rye straw, × 12.

or tapering slightly at tip, rounded at both ends or slightly flattened at the hilum, hilum not protruding; produced singly or 1-catenate associated or not with short connecting cell, occasionally first spore produced is large, 120–165 μ, yellow to light golden-brown, producing a short secondary, apical conidiophore and smaller secondary conidia; typically germinating from any segment but one or two germ tubes first appearing from end segments. When the spores germinate on glass, appressorium-like bodies are formed at the end of the germ tubes.

Single spore cultures on potato-cerulose agar produced dark gray loose-growing colonies topped by scattered white tufts at the center

and small white flecks at the margin. In either case, the mycelium from ascospores and conidia consisted of sterile hyphae similar in characteristics.

Habitat: Parasitic on *Secale cereale* L. collected at Madison, Wisconsin, May 1, 1948 (Type); Hancock, Endeavor, Arena, and Poynette, Wisconsin, May 19, 1948.



FIGS. 20–28. Drawings of nuclear cycle of *Pyrenophora secalis*, × 1290. 20–22. Successive divisions occurring in the formation of the 8-nucleate ascus before free spore differentiation. 23. Differentiation of cytoplasm forming biseriate arrangement of nuclei. 24. Uninucleate ascospores at free spore differentiation. 25. Stages in the development of the multinucleate ascospores. 26. Nuclear condition of a germinating multinucleate ascospore. 27. Multinucleate mycelium. 28. Multinucleate mature conidium.

Portions of the type specimen collected at Madison, Wisconsin, are deposited in the mycological herbarium of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland; New York Botanical Garden, Bronx, New York; Harvard University, Cambridge, Massachusetts; and University of Wisconsin, Madison, Wisconsin.

There seemingly have been four species of *Pyrenophora* described on rye. Exsiccatus materials of these species; *P. relicina* (Funk)

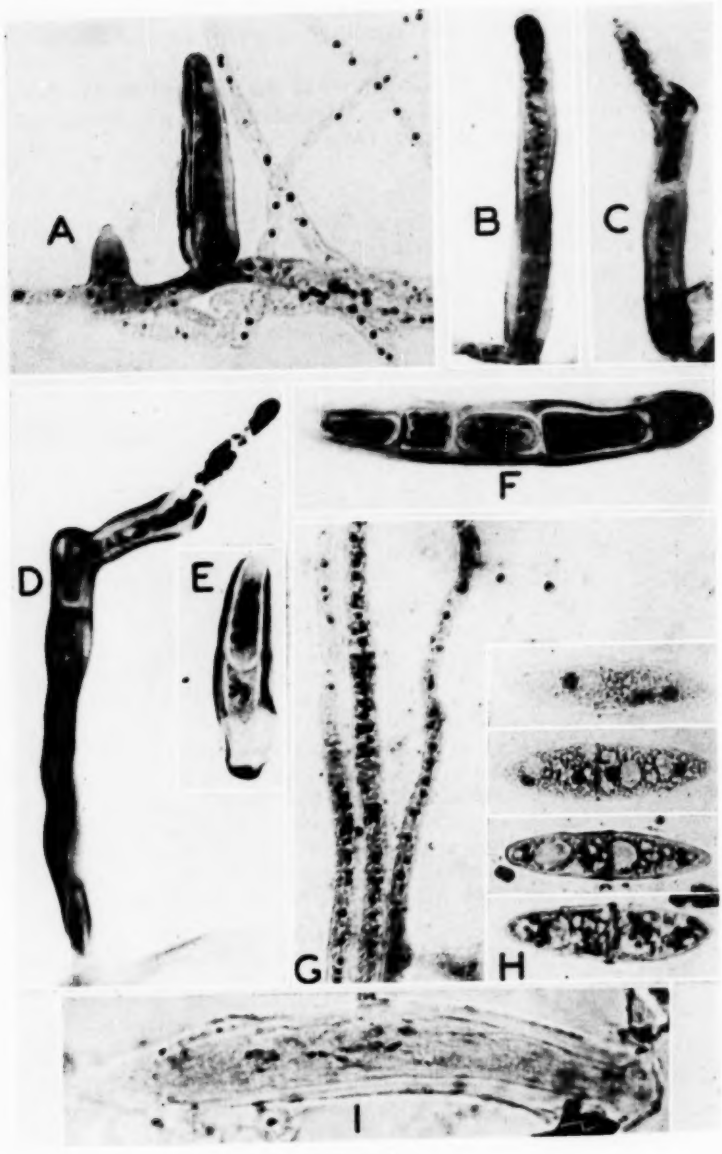


FIG. 29.

Sacc., *P. trichostroma* (Fr.) Sacc., *P. phaeocomes* (Reb.) Sacc., and *P. sarcocystis* B. & C. deposited in the U.S.D.A. herbarium were studied. From published descriptions and exsiccatus material studied, these species differ considerably in morphological measurements from *Pyrenophora secalis*. Descriptions of *Pyrenophora* of the other grass hosts did not reveal a special resembling *P. secalis*.

So far as the authors have been able to determine, only one species, *Helminthosporium tuberosum* Atk. has been described for rye. *H. tuberosum* differs from *H. secalis* in size, septation and spore color. *H. secalis* most closely resembles *H. catenarium* Drechsler described from *Cinna arundinaceae* L. because of its catenate tendency and spore size but differs mainly in mode of germination, thicker spore wall and slightly less tapering of the spore and the formation of a dark instead of white mycelial mass in culture.

The nuclear condition of the spores and mycelium was investigated. A study of stained germinating ascospores indicated that the cells of mature spores were multinucleate (FIG. 25). It appeared desirable, therefore, to determine the stage in spore development at which the multinucleate condition occurred. Stained smears of asci and ascospores showed that the young asci were uninucleate before spore differentiation (FIGS. 20–23). Biseriate arrangement of the nuclei was clearly differentiated at the eight nucleate stage when the cytoplasm was differentiated as two distinct strands streaming the length of the ascus (FIGS. 23, 29I). At spore differentiation the spores were uninucleate (FIG. 24). This nucleus divided and the daughter nuclei moved to opposite ends of the spore prior to formation of the first transverse septum. Each of these daughter nuclei divided and moved to the opposite ends of the cell prior to formation of the second and third septa. The multinucleate condition developed by further divisions of the existing nuclei after the spores were 3-septate. One to six nuclei were

FIG. 29. Photomicrographs of the nuclear cycle of *Pyrenophora secalis*, × 880. A. Formation of young multinucleate conidiophore. B. Movement of nuclei to apical end of the conidiophore prior to conidium formation. C, D. Multinucleate older conidiophores with conidia forming. E, F. Multinucleate young and mature conidia. G. Multinucleate mycelium. H. 2-nucleate, 4-nucleate, 4–8 nucleate division, and multinucleate ascospores. I. Ascus 4–8-nucleate stage showing division figures and spindle orientation.

observed per cell in mature ascospores (FIGS. 25, 29H). The mycelium was predominately multinucleate, ranging from one to six nuclei per cell (FIGS. 26, 27, 29G). Several nuclei moved out into the young conidiophore early in its formation (FIG. 29A), and the conidiophore remained multinucleate to maturity (FIG. 29A–D). Nuclei of the end segment of the conidiophore moved to the distal end prior to formation of the conidium (FIG. 29B) and moved into the conidium on its formation (FIG. 29C, D). The conidia were multinucleate during all stages of development (FIG. 29E, F).

SUMMARY

Two new species of *Pyrenophora: Pyrenophora alternarina* and *P. secalis,* with their conidial stages, are described.

Pyrenophora alternarina is suggested as probably the first reference connecting the genus *Pyrenophora* with *Alternaria.*

Pyrenophora secalis, with an *Helminthosporium* conidial stage, produces a severe leaf spot of rye seedlings. The developmental nuclear cycle of the fungus to the formation of multinucleate ascospores and conidia is described and figured.

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PHYCOMYCETES FROM THE DOUGLAS LAKE REGION OF NORTHERN MICHIGAN

F. K. Sparrow [1]

(with 1 figure)

During the summer of 1948 an investigation of the phycomycetous flora of the Douglas Lake Region of Northern Michigan, Cheboygan County, was initiated. While the work centered for the most part around the Biological Station of the University of Michigan situated on Douglas Lake, collections were also made in the Cross Village area in Emmet County on Lake Michigan, and from the numerous beach pools on Mackinac Island, Mackinac County. The investigation was continued in roughly the same area during the summer of 1950. The writer is responsible for the final identification of all fungi listed except certain of the saprolegniaceous forms which were kindly contributed by Terry W. Johnson, Jr.

The methods used in the collection and isolation of these fungi were the standard ones employed by workers in the field of aquatic molds (Couch, **1939**, Sparrow, **1943**). Those interested in specific processes are referred to the papers cited.

Fungi Collected

chytridiales

Olpidium pendulum Zopf.—In pine pollen, Douglas Lake, July 14, 1950.

Rozellopsis septigena (Fischer) Karling.—Parasitic in hyphae of *Saprolegnia* sp., Douglas Lake, July 10, 1950.

[1] Contribution No. 393 from the Botany Department, and from the Biological Station, University of Michigan.

MICROMYCES ZYGOGONII Dangeard.—Parasitic in vegetative filaments of *Mougeotia genuflexa*. Abundant in shallow pools in Smith's Bog, Cheboygan Co., July 6, 1948.

This is the third record of the occurrence of this fungus in the United States. The numerous shallow pools in Smith's Bog are rich in various algae early in the season and a careful survey should yield a variety of fungous parasites.

Phlyctidium tenue sp. nov.

Sporangium sessile, hemisphaericum, 15–16 μ diametro, 12–14 μ altum; membrana laevi, pellucida, pro majore parte tenui sed in parte infima distincte incrassata et basem poculiformem formante. Haustorium gracile dupliciter membranaceum, plerumque rectum simplex, diametro uniforme, longitudine variabile. Zoosporae numerosae, oblongae, 0.5 μ latae, 2.5 μ longae, globulo medio pellucido refractivo et flagello singulo longo postico praeditae, per poram singulam apicalem emissae. Post emittionem zoosporarum collapsae sunt sporangii membranae. Sporae perdurantes sessiles, vel globosae, 13–15 μ diametro, vel ellipsoideae, 10 μ latae, 12 μ longae, possibiliter a conjuctione sexuali formatae, thallo contributorio (3 μ diametro) annexo; membrana paululo incrassata laevi; globulo magno, medio, oleari; haustorio simplici endobiotico. Germinatio nondum observata est.

In cellulis vegetativis moribundis *Zygnematis* parasiticum in stagnis prope viam "South Lake Shore Boulevard," in insula "Mackinac," Michigan, 18 jul. 1948.

Sporangium sessile, somewhat hemispherical, 15–16 μ in diameter by 12–14 μ high; wall smooth, colorless, distinctly thickened on the lower part of the sporangium so as to form a cup-like base. Haustorium slender, double-contoured, usually straight, unbranched, isodiametric, of variable length. Zoospores numerous, oblong, 5 μ long by 2.5 μ wide, with a centric, colorless refractive globule and a single posterior flagellum 15 μ long; escaping through a single apical pore after which the sporangium wall collapses. Resting spores epibiotic, sessile, spherical, and 13–15 μ in diameter, or ellipsoidal and 10 $\times$ 12 μ, with a slightly thickened wall, large central colorless oil globule, and an unbranched endobiotic haustorium; possibly sexually formed, the adnate contributing thallus 3 μ in diameter; germination not observed.

On moribund vegetative cells of *Zygnema* sp., in beach pool by South Lake Shore Boulevard, near Grand Hotel, Mackinac Island, July 18, 1948.

Discharge of the zoospores occurs after the deliquescence of an imperceptible apical papilla (FIG. 1, a–d). They emerge passively

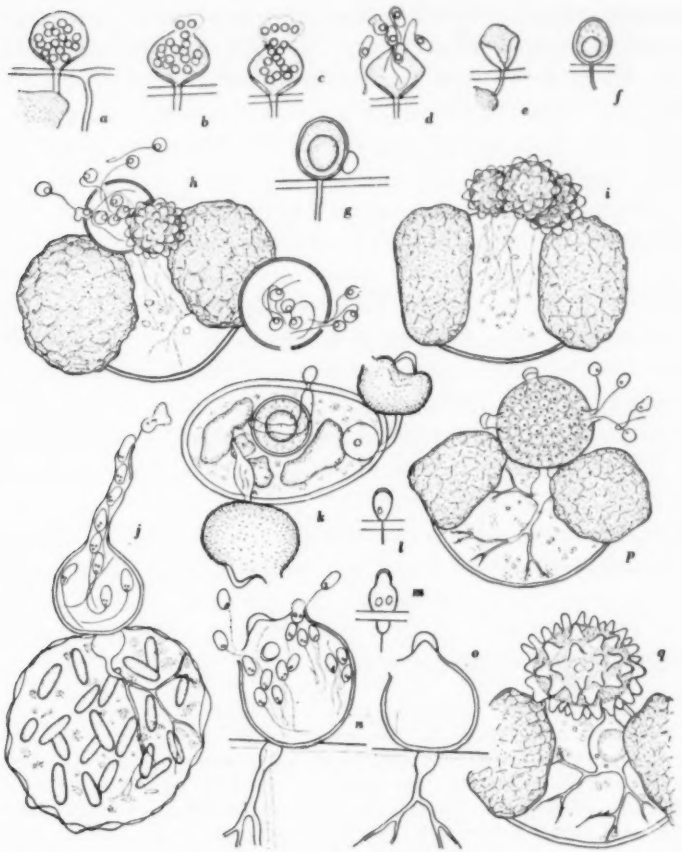

FIG. 1, *a–g*. *Phlyctidium tenue* on *Zygnema* sp.; *a–d*, stages in the discharge of the zoospores; *e*, empty sporangium showing fractured delicate wall; *f*, resting spore; *g*, resting spore with male (?) cell attached.

FIG. 1, *h*, *i*. *Rhizophydium bullatum* on pine pollen; *h*, two discharging sporangia and a resting spore; *i*, pollen grain with three resting spores.

FIG. 1, *j*. Discharging sporangium of *Phlyctochytrium longicollum* on cysts of *Euglena* sp.

FIG. 1, *k*. Zygospore of *Spirogyra* sp. bearing two empty sporangia and a resting spore of *Blyttiomyces spinulosis*.

FIG. 1, *l–o*. *Blyttiomyces laevis* on *Zygnema* sp.; *l*, *m*, early stages in thallus formation; *n*, discharging multiporous sporangium; *o*, empty sporangium.

FIG. 1, *p*, *q*. *Phlyctochytrium papillatum* on pine pollen; *p*, discharging sporangium; *q*, resting spore.

FIG. 1, *o*. × 842; all others × 337.

and only assume motility after a period of quiescence in the vicinity of the sporangium (FIG. 1, d). Not only does the sporangium wall, except for its rigid base, collapse after discharge, but it may also occasionally fragment (FIG. 1, e). Thick-walled resting spores with (FIG. 1, g) and without (FIG. 1, f) adnate male thalli have been found.

The species differs from all others of the genus in the basal thickening of the otherwise delicate sporangium wall.

SEPTOSPERMA RHIZOPHIDII Whiffen.—Parasitic on cysts of a microscopic animal, Bryant's Bog, Douglas Lake, July 30, 1950.

RHIZOPHYDIUM CHAETIFERUM Sparrow.—Saprophytic on vegetative filaments of *Spirogyra* sp. in same beach pool as *Phlyctidium tenue*, July 18, 1948; on *Oedogonium* sp., Smith's Bog, June 26, 1950. *Spirogyra* has not heretofore been reported as a substratum for this species.

RHIZOPHYDIUM SPHAEROTHECA Zopf.—On pine pollen bait, soil from beneath mat of *Polytrichum piliferum* along trail to Grapevine Point, Biological Station, Douglas Lake, June 23, 1948.

Rhizophydium bullatum sp. nov.

Sporangia sessilia, aggregata, globosa, membranis duplicibus, diametro inter 6.6 μ et 15.4 μ variantia. Systema rhizoidale tenue, multipliciter ramosum. Zoosporae numerosae, sphaericae, 3 μ diametientes, globula singula basali pellucida et flagello longo posteriore praeditae, per poras 2–5 sessiles liberatae. Sporae perdurantes sphaericae, 6.6–11 μ diametro, sessiles, aggregatae vel singulatim dispersae; membrana incrassata fusca dense bullata vel mamillata; globula singula. Germinatio nondum observata.

Parasiticum in polline *Pinus* infecto a sporis ex humo ad locum "Grape Vine Point" dictum, Stationem Biologicam Univ. Michiganensis, Cheboygan Co., Michigan, Jun. 28, 1948.

Sporangia sessile, in groups, spherical, with a smooth, double-contoured wall, varying from 6.6–15.4 μ in diameter. Rhizoidal system very delicate, much branched. Zoospores numerous, spherical, 3 μ in diameter, with a single basal colorless globule and long posterior flagellum; escaping through 2–5 sessile pores. Resting spores spherical, 6.6–11 μ in diameter, sessile, occurring in groups or occasionally singly, with a thickened brownish wall beset with coarse bullations; contents bearing a single large globule; germination not observed.

Parasitic on pine pollen bait; soil from bank along Grapevine Point trail, Biological Station, University of Michigan, Cheboygan Co., Michigan, June 28, 1948.

In the shape and size of its sporangia (FIG. 1, h) *R. bullatum* resembles *R. sphaerotheca* Zopf. No resting spores have ever been definitely associated with Zopf's species whereas in the new species they (FIG. 1, h, i) were abundantly and quickly formed. The resting spores of *Rhizophydium Closterii* Karling bear a strong resemblance to those of the present species. The sporangia of the two, however, are quite different, being somewhat spherical to pyriform with a single large apical pore in Karling's species and spherical and multiporous in the Michigan fungus.

It is entirely possible that there are a number of spherical multiporous sporangial types which differ only in the character of their resting spore.

RHIZOPHYDIUM POLLINIS-PINI (Braun) Zopf.—On pine pollen, Smith's Bog, near Biological Station, June 25, 1948; Douglas Lake, June 27, 1948.

RHIZOPHYDIUM KERATINOPHILUM Karling.—Saprophytic on human hair used as bait, algal debris from Smith's Bog, near Biological Station, August 10, 1950.

RHIZOPHYDIUM (?) CLOSTERII Karling. On *Closterium* sp., Smith's Bog, vicinity of the Biological Station, July 7, 1948.

Only the zoosporangia were found. These conformed so closely, however, in every feature to those described by Karling (**1946**), including the constant presence of a slight endobiotic, subsporangial swelling of the rhizoidal axis that the identification is probably correct.

Phlyctochytrium longicollum sp. nov.

Sporangium sessile, sphaericum, laeve, 17–20 μ diametro, maturitate tubum efferentem cylindricum rectum vel subflexuosum 18–20 μ longum 5 μ crassum ferens. Systema rhizoidale tenue ramosissimum ex axe singulo primario ad basin apophysis endobiotici infrasporangialis oriens. Zoosporae ellipsoidales vel paene oblongae, 3.3 μ longae, 2.2 μ latae, globulis pluribus minutis hyalinis refractivis et flagello longo posteriore praeditae. Sporae perdurantes non observatae.

Parasiticum in *Euglena* sp. dormiente ad locum "Nichols Bog" dictum prope Stationem Biologicam Universitatis Michiganensis, Cheboygan Co., Michigan, Julio 12, 1948.

Sporangium sessile, spherical, smooth-walled, 17–20 μ in diameter, bearing at maturity a cylindrical, straight or somewhat undulating discharge tube 18–20 μ in length by 5 μ in diameter. Rhizoids delicate, much branched, arising from a single main axis at the base of a spherical, 8 μ in diameter, subsporangial, endobiotic apophysis. Zoospores ellipsoidal to nearly oblong, 3.3 × 2.2 μ, with several minute, basal, colorless, refractive granules and a long posterior flagellum. Resting spores not observed.

Parasitic on encysted *Euglena* sp., Nichols Bog, Cheboygan Co., Michigan, July 12, 1948.

In its general appearance *P. longicollum* strongly resembles *Rhizidiomyces apophysatus* Zopf. Only after a study of the method of discharge of the zoospores and their structure (FIG. 1, j) were the true affinities of the fungus recognized. No other species of the genus forms a long discharge tube.

Phlyctochytrium papillatum sp. nov.

Sporangium sessile, sphaericum 23–25 μ diametro, papillis emittientibus 3–5 valde elevatis 4 μ diametientibus praeditum. Systema rhizoidale modice crassum ramosum, ex base apophysis infrasporangialis subovoidei endobiotici oriens. Zoosporae sphaericae, 4 μ diametro, globulo singulo minuto pellucido eccentrico et flagello longo praeditae, per 3–5 poras a deliquescentia papillarum emittientium formatas emissae. Sporae purdurantes sessiles subglobosae, 27 μ diametro, membrana modice crassa, papillis numerosis crassis obtusis pellucidis induta. Systema rhizoidale ei sporangii simile. Germinatio nondum observata est.

Parasiticum in polline *Pinus* cum *Rhizophidio bullato*, in sole in ripis secus viam "Grapevine Point Trail" prope Stationem Biologicam Universitatis Michiganensis, Cheboygan Co., Michigan, Jun. 28, 1948.

Sporangium sessile, spherical, 23–25 μ in diameter, with 3–5 distinctly elevated, 4 μ in diameter discharge papillae. Rhizoidal system moderately coarse, branched, arising from the base of a subsporangial endobiotic, somewhat ovoid apophysis. Zoospores spherical, 4 μ in diameter, with a single minute, colorless eccentric globule and a long flagellum; emerging through 3–5 pores formed upon the deliquescence of the discharge papillae. Resting spores sessile, subspherical, 27 μ in diameter, with a moderately thick wall beset with numerous stout, blunt, colorless papillae; rhizoidal system like that of the sporangium; germination not observed.

Parasitic on pine pollen in association with *Rhizophidium bullatum;* in soil from bank along Grapevine Point Trail, Biological Station, University of Michigan, Cheboygan Co., Mich., June 28, 1948.

The species, while resembling *P. synchytrii* Kohler, differs from it in the ovoid shape of the apophysis (FIG. 1, p) and papillate resting spores (FIG. 1, q).

PHLYCTOCHYTRIUM PLANICORNE Atkinson.—On oogonia of *Oedogonium* sp., Smith's Bog, June 29, 1950. The species has not heretofore been reported on the oogonia of *Oedogonium.*

BLYTTIOMYCES SPINULOSUS (Blytt) Bartsch.—Parasitic on zygospores of *Spirogyra* sp., beach pool, South Shore Lake Boulevard near Grand Hotel, Mackinac Island, July 18, 1948.

There is evidence for believing that a certain degree of host specificity is shown by this species. In the collection of algae made at this site there were many thousands of ellipsoidal and spherical zygospores intermixed. Although there was heavy infection of the ellipsoidal ones (FIG. 1, k), not a single spherical zygospore (presumably belonging to another species) was attacked. Although *Blyttiomyces spinulosis* has been recorded at least five times on continental Europe it has apparently been recorded only once before in America, namely, by Bartsch (**1939**) from Wisconsin. A report of its occurrence in epidemic proportions in Cuba is reported by Sparrow (**1952**).

Blyttiomyces laevis sp. nov.

Sporangium sessile, ovoideum, 14–17 μ longum, 12–16 μ crassum, laeve, apiculatum, apiculo prominulo, convexo, membrana incrassata, 2.5–3.5 μ alto, 3–4 μ diametro. Systema rhizoidale crassum, sparsim ramosum ad apophysin parvum subsporangiale adnatum. Zoosporae numerosae subovoideae, diametro 3 μ, granulis 1 vel pellucidis refractivis et flagello longo posteriore praeditae, ex poris 1–3 subapicalibus emittientes. Sporae perdurantes hucusque ignotae.

Parasiticum in *Zygnema* sp., in loco "Nichol's Bog" dicto prope Stationem Biologicum, Univ. Michiganensis, Cheboygan Co., Jul. 12, 1948.

Sporangium sessile, ovate, smooth-walled, 14–17 μ high by 12–16 μ in diameter, with a subapical prominent thick-walled, strongly convex apiculus 2.5–3.5 μ high by 3–4 μ in diameter. Rhizoids

coarse, sparsely branched, arising from a small subsporangial apophysis. Zoospores numerous, slightly ovoid, 3 μ in diameter with one or two minute, colorless, refractive granules and a long posterior flagellum; escaping through 1–3 subapical pores. Resting spore not observed.

Parasitic on filaments of *Zygnema* sp., Nichols Bog, vicinity of University of Michigan Biological Station, July 12, 1948.

A study of the development and structure of this fungus indicates that while superficially resembling a species of *Phlyctochytrium* it is more closely allied to the monotypic genus *Blyttiomyces*.

The zoospore upon coming to rest on the algal wall encysts and forms a delicate, unbranched penetration tube. After this slender structure has elongated somewhat, that part just beneath the host wall starts to expand to form the rudiment of the apophysis (FIG. 1, l, m). Meanwhile, the body of the spore itself initiates elongation. Almost from the first there is apparent a narrow distal arc of material which is more refractive and thicker than the remainder of the thallus wall. As gradual expansion of the main body of the thallus continues, this apical portion remains relatively unchanged in size. It does, however, become more convex and thicker walled (FIG. 1, m). Although resembling at maturity the discharge papilla of a chytrid sporangium, it is in reality the apiculus of the sporangium (FIG. 1, n, o). The laying down early in the development of the thallus of the rudiment of the apiculus closely parallels the formation of the conspicuous apical spine of the curious chytrid *Obelidium mucronatum*. Here, it will be recalled (Sparrow, **1938**), spine formation is initiated even before rhizoids are produced. Further developmental features and maturation of the zoospores are not notably different from other members of the Phlyctidiaceae. One to three subapical discharge papillae form which upon deliquescing allow the zoospores to escape.

Little broadening of the generic concept of *Blyttiomyces* is necessary in order to include the present fungus. Bartsch (**1939**) states that the intramatrical portion of the thallus includes ". . . two, rarely three, apophyses . . ." and that the sporangium is provided with a ". . . subapical exit pore. . . ." In both Michigan and Cuban material (Sparrow, **1952**) of what is otherwise typical

B. spinulosis, however, many examples of thalli with only a single apophysis were observed (FIG. 1, k). Further, the number of discharge pores, in the light of the situation prevailing in other, probably related genera (as, for example, *Rhizophydium*), does not seem worth retaining as a character of generic rank.

Another multiporous epibiotic chytrid, probably related to *Blyttiomyces* was encountered on *Netrium* sp. collected in Bryant's Bog, July 6, 1950. Its sporangia were spherical, 12–13 μ in diameter, the apiculus 3 μ in diameter, the wall minutely spiny and the endobiotic apophysis from which a delicate, sparingly branched rhizoidal system emerged, 3 μ in diameter. One to three discharge pores were formed. Further observations on the zoospores and resting spores will be necessary before this undoubtedly new species can be adequately described.

ENTOPHLYCTIS CONFERVAE-GLOMERATAE (Cienkowski) Sparrow.—In moribund filaments of *Vaucheria* sp., beach pool near "Pontiac's Lookout," Mackinac Island, July 18, 1948.

DIPLOPHLYCTIS INTESTINA (Schenk) Schroeter.—In moribund and dead internodal cells of *Nitella* sp., Bessey Creek, July 2, 1948; July 3, 1950.

DIPLOPHLYCTIS LAEVIS Sparrow.—Dead internodal cells of *Nitella*, July 3, 1950.

This species has heretofore been known only on *Cladophora*.

See, however, Richards (Trans. British Mycol. Soc. **34**: 483. 1951), where it is described in *Chara*.

RHIZOPHLYCTIS PETERSENII Sparrow.—On cellophane "bait," in water culture with soil from Grapevine Point, Douglas Lake, June 23, 1948; Smith's Bog, July 7, 1950.

RHIZOCLOSMATIUM GLOBOSUM H. E. Peterson.—Within the exuviae of various aquatic insects, beach pool, Cross Village, Emmet Co., July 5, 1948; beach pool, South Lake Shore Boulevard, Mackinac Island, July 18, 1948.

RHIZOCLOSMATIUM AURANTIACUM Sparrow.—Within exuviae of aquatic insects, beach pool, Cross Village, Emmet County, July 5, 1948; beach pools, near "Pontiac's Lookout," and "Wishing Well," Mackinac Island, July 18, 1948.

ASTEROPHLYCTIS SARCOPTOIDES H. E. Petersen.—Within the exuviae of various aquatic insects, beach pool, Cross Village, Emmet Co., July 5, 1948; July 20, 1950.

The lack of sexuality preceding the formation of the resting spore of this distinctive chytrid, as demonstrated by Antikajian (**1949**), is hardly sufficient grounds for suppression of the genus, as she advocates.

SIPHONARIA VARIABILIS H. E. Petersen.—Within the exuviae of various aquatic insects, beach pool, Cheboygan City bathing beach, Mackinac Straits, August 10, 1948.

CLADOCHYTRIUM REPLICATUM Karling.—Saprophytic on vegetable debris, beach pool near Grand Hotel, Mackinac Island, July 18, 1948.

PHYSODERMA MENYANTHIS de Bary.[2]—Parasitic on leaves of *Menyanthes trifoliata,* Mud Lake Bog, Cheboygan Co., July 8, 1948; Bryant's Bog, Douglas Lake, July 20, 1950; Black Lake shoreline, August 2, 1950.

CHYTRIDIUM SCHENKII (Schenk) Scherffel.—On vegetative filaments of *Oedogonium* sp., beach pool, Cross Village, Emmet Co., July 20, 1950.

ENDOCHYTRIUM OPERCULATUM (de Wild.) Karling.—In decayed maize stem, beach pool, Cross Village, Emmet Co., July 20, 1950.

NOWAKOWSKIELLA ELEGANS (Nowak.) Schroeter.—In decaying maize stem, Bessey Creek, August 9, 1950.

NOWAKOWSKIELLA HEMISPHAEROSPORA Shanor.—On cellophane "bait" in water culture from beach pool near "Pontiac's Lookout," Mackinac Island, July 18, 1948; Bessey Creek, July 27, 1950 (coll. M. C. Walsh).

[2] The family Physodermataceae has been proposed (Sparrow, 1943: 74) but not given the required Latin diagnosis. This is now presented.

Physodermataceae fam. nov.

Fungi chytridiales thallis biformibus, primo monocentrico systema endobioticum et sporangium epibioticum membrana tenui indutum sistante, alio polycentrico, omnino endobiotico, sporas numerosas perdurantes et cellulas turbinatas ferente. Sporae perdurantes germinatione singulatim zoosporangium membrana tenui indutum formantes. Parasitici in plantis Angiospermis.—Genus typicum *Physoderma* Wallr.

BLASTOCLADIALES

CATENARIA ANGUILLULAE Sorokin.—Parasitic in the eggs of an undetermined microscopic animal, beach pool, Cross Village, Emmet Co., July 20, 1950; cast skin of snake used as "bait," Bryant's Bog, August 3, 1950 (coll. M. C. Walsh).

MONOBLEPHARIDALES

MONOBLEPHARIS MACRANDRA (Lagerh.) Woronin.—Submerged twigs of White Birch, stream running out of Carp Lake, Cheboygan Co., July 5, 1948 (coll. M. C. Walsh).

This appears to be the fourth record of this fungus from the United States and the first from Michigan.

SAPROLEGNIALES [3]

SAPROLEGNIA PARASITICA Coker.—On various kinds of small fish, Douglas Lake, June 17, 1948; Maple River near Pellston, July 2, 1948; Carp Lake, July 5, 1948, all Cheboygan Co.; Round Lake, Emmet Co., July 7, 1950 (coll. F. K. S.).

SAPROLEGNIA DICLINA Humphrey.—On hempseed "bait," Douglas Lake, June 21, 1948.

SAPROLEGNIA FERAX Thuret.—On hempseed "bait," Douglas Lake, June 21, 1948; Maple River near Pellston, July 2, 1948; Nichols Bog, vicinity of Biological Station, July 11, 1948; Sturgeon Bay, Lake Michigan, June 30, 1948; Cheboygan River, 3½ miles south of Cheboygan, July 11, 1948.

SAPROLEGNIA MONOICA var. GLOMERATA Tiesenhausen.—Hempseed "bait," stream 6 miles E. of Biological Station, July 24, 1948.

SAPROLEGNIA LITORALIS Coker.—Hempseed "bait," Bryant's Bog, June 25, 1948.

SAPROLEGNIA MIXTA de Bary.—Hempseed "bait," Reese's Bog, June 28; Mud Lake Bog, Cheboygan Co., July 24, 1948.

ACHLYA RACEMOSA Hildebrand.—Hempseed "bait," Douglas Lake, June 25, 1948; Carp Creek, Cheboygan Co., July 11, 1948.

[3] Collections and identifications made by Terry W. Johnson, Jr. Records of species from Mackinac Island published by Johnson (**1950**) are not included here.

ACHLYA AMERICANA Humphrey.—Hempseed bait; widely distributed throughout the region. This species was recovered 87 times from 39 aquatic and semiaquatic sites.

ACHLYA IMPERFECTA Coker.—Hempseed "bait," Douglas Lake, June 25, 1948.

ACHLYA SUBTERRANEA Coker and Braxton.—Hempseed "bait," soil from south shore of Douglas Lake, June 5, 1948.

This is the first record of the occurrence of this species since its discovery in 1926.

ACHLYA KLEBSIANA Pieters.—Hempseed "bait," Reese's Bog, June 28, 1948.

Achlya michiganensis Johnson (**1950**), described from Mackinac Island is now considered by him to be synonymous with this species.

ACHLYA TRELEASEANA (Humphrey) Kauffman.—Hempseed "bait," Reese's Bog, June 28, Bryant's Bog, June 25, Nichol's Bog, July 11 and Smith's Bog, July 12, 1948.

A common bog-inhabiting species in both Northern and Southern Michigan.

ACHLYA COLORATA Pringsheim.—Hempseed "bait," creek bed, two miles southwest of Cheboygan, July 1, 1948.

ISOACHLYA TORULIODES Kauffman.—Hempseed "bait," Munro Lake, Cheboygan Co., July 2, 1948; ditch, mile north of Brutus on Highway U. S. No. 31, July 16, 1948.

DICTYUCHUS MONOSPORUS Leitgeb.—Hempseed "bait," Bryant's Bog, June 25, and creek two miles southwest of Cheboygan, July 1, 1948.

DICTYUCHUS ANOMALUS Nagai.—Hempseed "bait," Indian River, west of Highway U. S. No. 27, July 13, 1948.

BREVILEGNIA SUBCLAVATA Couch.—Hempseed "bait," south shore of Sturgeon Bay, Lake Michigan, Emmet Co., June 30, 1948.

THRAUSTOTHECA CLAVATA (de Bary) Humphrey.—Southeast point of Munro Lake, Cheboygan Co., July 2; Crooked Lake, Alanson, July 16, 1948.

APHANOMYCES LAEVIS de Bary.—In exuviae of chironomids, Douglas Lake, June 17, 1948 (ident. F. K. S.).

An exceedingly common species in exuviae.

ENTOMOPHTHORALES

CONIDIOBOLUS VILLOSUS Martin.—Saprophytic on cast skin of snake used as "bait" in water culture of soil and rotten wood, Smith's Bog, June 29, 1950 (collected and isolated by M. C. Walsh).

No critical study has been given, as yet, to the other members of this order which are undoubtedly present in the region. At least one species of *Entomophthora* is common on insects in the boat houses of the Biological Station.

ANCYLISTIS CLOSTERII Pfitzer.—Parasitic in *Closterium* sp., Bryant's Bog, August 17, 1950.

Active formation and discharge of the conidia were taking place. These processes were exactly as described by Berdan (**1938**).

MUCORALES

ENDOGONE PISIFORMIS Link.—On *Sphagnum*, Bryant's Bog, July 20, 1948.

PERONOSPORALES

ZOOPHAGUS INSIDIANS Sommerstorff.—Parasitizing rotifers of the genus *Rattulus*, Bryant's Bog, July 6, 1950.

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SYNONYMY OF TILLETIA HORRIDA AND NEOVOSSIA BARCLAYANA [1]

E. C. TULLIS AND A. G. JOHNSON [2]

(WITH 1 FIGURE)

Kernel smut of rice was reported first from Japan in 1896 by Takahashi (**26**), who named the causal fungus *Tilletia horrida* Tak. In 1899, Anderson (**1, 2**) reported the occurrence of a smut on rice in South Carolina, which was identified by Earle as *T. corona* Scrib. Anderson (**1, 2**), on the basis of comparing the descriptions of *T. horrida* and *T. corona,* suggested that the two were identical. Earle (**11**), in 1899, stated that he had not intended to express a final opinion to Anderson, and on seeing the description of *T. horrida* from Japan, concluded that the smut on rice from South Carolina was *T. horrida.* With this Anderson agreed (**3**). Pammel *et al.* (**19**) erroneously referred to this rice smut as *T. rotundata* (Arth.) Ell. & Ev.

It is of special interest to note here that Anderson had suggested that *Tilletia horrida* and *T. corona* were probably identical, and also that, shortly thereafter, Massee (**16,** p. 156), in revising the genus *Tilletia,* excluded *T. corona* from that genus and placed it in the genus *Neovossia.* More recently Padwick and Azmatullah Khan (**18,** p. 2) have placed the rice kernel smut fungus, *T. horrida,* in the genus *Neovossia,* as *N. horrida* (Tak.) Padw. and A. Khan. As there are certain points of difference between *Tilletia* and *Neovossia,* particularly in the germination of the chlamydo-

[1] Investigations conducted in cooperation with the Texas, Arkansas, and Louisiana Agricultural Experiment Stations (Texas Agricultural Experiment Station Technical Article No. 1523).

[2] Pathologist and collaborator, formerly Pathologist and Principal Pathologist, respectively, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

spores, a summary of the literature on germination of the chlamydospores of *T. horrida* and *N. barclayana* Brefeld is presented.

Germination of the chlamydospores of *Tilletia horrida* has been reported by Takahashi (**26**), Teng (**27**), Vanderpool (**31**), Cralley (**10**), Wei (**33**), and Lin (**15**). Takahashi (**26**), in connection with the original description of *T. horrida* in 1896, reported that the chlamydospores germinated in water and that the promycelia produced at their tips 10–20 filiform sporidia, 38–53 μ long. He also noted the absence of conjugation between these sporidia. No secondary sporidia were noted.

In 1931, Teng (**27**) described and illustrated the germination of the chlamydospores more fully. He reported germination in water, only near the surface where oxygen supply was abundant. He reported terminally produced primary sporidia, 2×35–$60\ \mu$. He stated: ". . . some of the sporidia may be seen to lie in pairs . . . ," and the legend for figure "d" reads "Fused sporidia sending out mycelial threads and forming conidia." There is, however, no evidence, in either the text or the illustrations, that real conjugation occurred. His illustrations show obclavate-allantoid secondary sporidia produced on the primary sporidia and also on hyphae produced by the latter.

In 1932, Vanderpool (**31**) reported using Bodine's (**4**) double-plate method successfully in germinating the chlamydospores of *Tilletia horrida*, and also reported the forceful discharge of the secondary sporidia.

In 1934, Cralley (**10**) reported results of germinating the chlamydospores of *Tilletia horrida* by the double-plate method. He noted non-septate promycelia, 5–8×10–$300\ \mu$, with 30–50 filiform primary sporidia, 2×35–$55\ \mu$, produced terminally in whorls. No conjugation of primary sporidia was observed. He noted abundant, curved-obclavate secondary sporidia, produced either on the germinating primary sporidia or on hyphae produced by them. He also noted that the secondary sporidia were forcibly discharged.

Wei (**33**) reported germination of the chlamydospores in water and illustrated simple and branched promycelia.

Lin (**15**) reported 4 to 5 months dormancy in the chlamydospores, and that after such a period, light and temperature influ-

enced germination. He reported secondary sporidia formed singly on hyphae produced by the primary sporidia.

A number of investigators (**2, 6, 13, 21, 32**) have reported unsuccessful attempts to germinate the chlamydospores of *Tilletia horrida*. Possibly the spores had not aged sufficiently.

The germination of the chlamydospores of *Neovossia barclayana* apparently has been observed only by Brefeld (**5**), who described and illustrated their germination in connection with the description of the species. He secured germination in water even after 3 years' storage, but not after 4 years' storage. On germination of the chlamydospores, thick, non-septate promycelia produced dense heads of very narrowly obclavate, primary sporidia that do not conjugate. The tips of the promycelia were simple or branched. The primary sporidia germinated and produced obclavate, straight or curved secondary conidia in the air, or they germinated in nutrient solutions and produced a mycelium, which in turn produced two kinds of secondary sporidia in the air. Some of these were like those referred to above, and the others were very narrowly obclavate, closely resembling the primary sporidia, except that they apparently were slightly thicker and somewhat shorter. There were also other secondary sporidia more or less intermediate between the two kinds just described.

Tilletia horrida was found on rice in Arkansas in 1926 (**35**), again in 1928, and 1929. Since then, none has been found in Arkansas, and only a few smutted kernels have been found in Louisiana. However, in December, 1936, the senior writer (**30**) received smutted rice kernels from the 1936 crop from the rice-growing area southwest of Houston, Texas. No difficulty was experienced in germinating the chlamydospores from these smutted kernels on water agar and pure cultures of the smut were secured.

During the examination of these cultures it was observed that the mycelium from the primary sporidia produced secondary sporidia of the obclavate-allantoid type usually formed by species of *Tilletia* and previously described for *T. horrida*, and, in addition, filiform secondary sporidia similar to but shorter than the primary sporidia. Both kinds of secondary sporidia were borne singly on the mycelium.

An examination of descriptions of closely related genera showed that having two kinds of secondary sporidia was typical of the genus *Neovossia*, the type species of which is *N. moliniae* (Thuem.) Koern. (**14**). Other characters in which the genus *Neovossia* differs from *Tilletia* are as follows: In *Neovossia moliniae*, the type species, the hyaline envelope, in which the chlamydospores are borne singly, invests each spore and tapers out at the proximal end, forming a conspicuous appendage. In *Tilletia* the hyaline envelope usually is present, but the remains of the sporiferous hypha usually are not evident. Occasionally, however, it can be seen, particularly on immature spores. In *Neovossia*, the primary sporidia are very numerous, 20 to 60 in number, form a distinct head, and do not conjugate, while in *Tilletia* the primary sporidia are distinctly fewer, about 12 in number, and frequently conjugate. In *Neovossia*, two kinds of secondary sporidia are formed on the mycelium that is produced by the germinating primary sporidia, while in *Tilletia* only one kind of secondary sporidium is formed.

On re-examining the rice kernel smut fungus and studying it further, it was found that it possessed all of the above characters of the genus *Neovossia* except that the appendages were more or less fragmentary and rather sparingly present on the fully matured spores. On immature spores, however, they were not uncommon. In fact, these attached mycelial fragments or fragmentary appendages were noted and illustrated by Anderson (**1, 2**) when this smut was first reported in the United States and are also referred to by Clinton (**9**) in his critical description of *Tilletia horrida*. Reyes (**21**) also noted and figured the rudimentary appendages on the immature chlamydospores.

Furthermore, it was found that when the spores of the kernel smut of rice germinated, they formed promycelia with dense heads of primary sporidia as described by Brefeld (**5**) for *Neovossia moliniae* and *N. barclayana* Brefeld. It was found also that the two kinds of secondary sporidia that were produced by the germinating rice smut spores agreed with those described and illustrated by Brefeld (**5**) for *N. barclayana* on *Pennisetum orientale* var. *triflorum* (Nees) Stapf (*P. triflorum* Nees) from India.

The size of the chlamydospores of the rice kernel smut fungus

and the sculpturing of their walls were found to be somewhat similar to those of *N. barclayana* Brefeld on *Pennisetum orientale* var. *triflorum* (*P. triflorum*) except that in the former the spores were somewhat larger and the sculpturing of their walls was distinctly coarser than in the latter. Furthermore, in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, there are two specimens of kernel smut on *P. setosum* (Swartz) L. Rich. (*P. purpurascens*), the one collected in Japan by K. Togashi, October 17, 1925, and the other collected in Korea by P. H. Dorsett and W. J. Morse, September 11, 1930. The Togashi specimen is labelled "*Tilletia corona* Scrib." and the Dorsett and Morse specimen is labelled *T. pennisetina* Syd. Both apparently are practically identical with *N. barclayana*, described by Brefeld from Simla, India, on *P. orientale* var. *triflorum* (*P. triflorum*), except that in the former the spores are a trifle larger and their walls slightly more coarsely sculptured than those of *N. barclayana* as originally described by Brefeld. These differences in chlamydospore characters, however, are much smaller than the differences between those spore characters of *Tilletia horrida* on rice and those of *T. horrida* on *Pennisetum alopecuroides* (L.) Spreng. and *P. glaucum* (L.) R. Br. from artificial inoculations referred to later. The same may be said of *T. pennisetina* Syd. as originally described by Sydow (**25**) on *P. alopecuroides* from China as compared with *T. horrida* from rice inoculated on *P. alopecuroides* and *P. glaucum* as referred to later.

Because of the many similarities of the kernel smut fungus of rice and *Neovossia barclayana* on *Pennisetum orientale* var. *triflorum* (*P. triflorum*) and also because a very similar smut had been found on *P. setosum* in Japan not far from the type locality of *Tilletia horrida* on rice, sporidia of the fungus from rice from Texas were used to inoculate rice and four species of *Pennisetum*, namely, *P. glaucum* (L.) R. Br., *P. setaceum* (Forsk.) Chiov. (*P. ruppellii* Steud.), *P. alopecuroides* (L.) Spreng., and *P. villosum* R. Br. For each of these, sterile seed with hulls removed were germinated in petri dishes on water agar and 25 young seedlings inoculated with secondary sporidia of *Tilletia horrida* from rice.

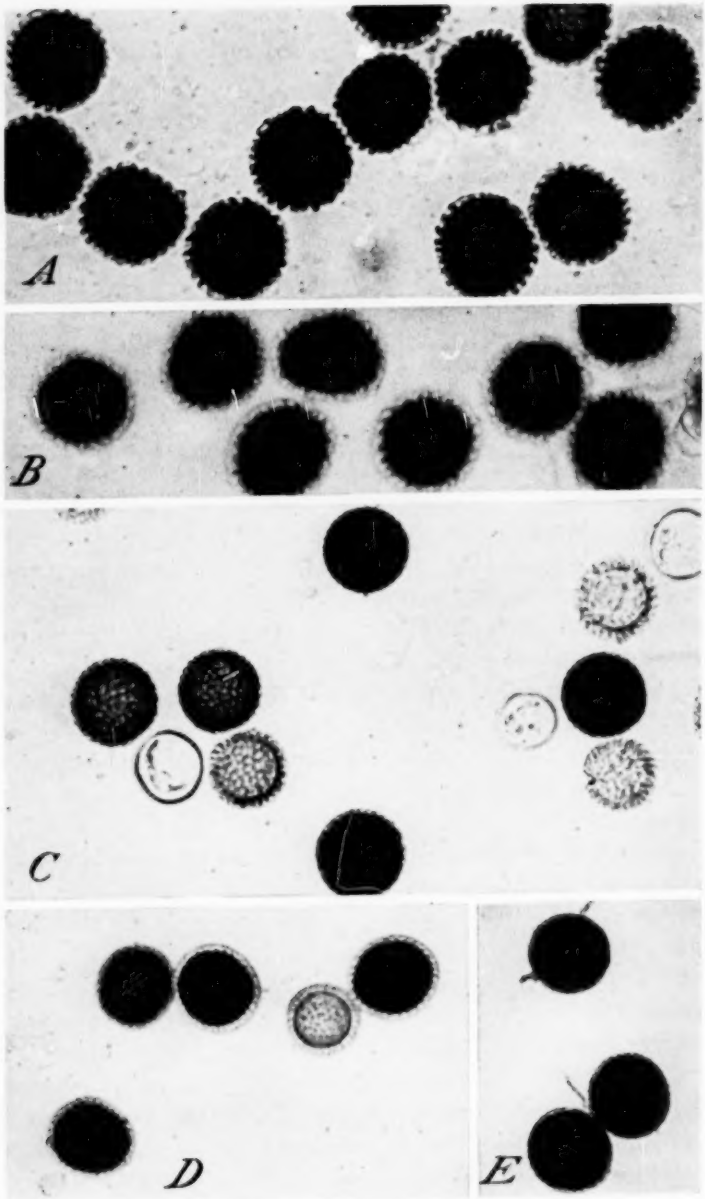

FIG. 1.

These seedlings were then transplanted into soil and grown to maturity in the greenhouse. Infection was secured on four plants of *P. glaucum* and two plants of *P. alopecuroides*. No smut was found on rice, *P. villosum* nor *P. setaceum*. The 25 uninoculated plants of each of these species remained free of smut. Photomicrographs of chlamydospores from rice (A, B), original inoculum, and from artificially inoculated *P. glaucum* (C) and *P. alopecuroides* (D) are shown in Fig. 1.

Through the kindness of J. A. Stevenson, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, a portion of the type material of *Neovossia barclayana* Brefeld on *Pennisetum orientale* var. *triflorum* (*P. triflorum*) was secured from Dr. E. Ulbrich, Botanisches Museum, Universität Berlin, Berlin-Dahlem, Germany, and a photomicrograph of chlamydospores from this is shown in FIG. 1, E.

A comparison was made of the spores from this type material with those secured from naturally-infected *Oryza sativa*, and the artificially inoculated *Pennisetum glaucum* and *P. alopecuroides*. Before measuring, and also before photographing, the spores were placed on one or one and one-half per cent water agar and left at least 24 hours in order to bring out more satisfactorily the markings of the epispore. The results of these spore measurements and also the spore measurements given by various investigators for *Tilletia horrida*, *T. pennisetina*, and *Neovossia barclayana* are given in TABLE I.

Chlamydospores from *Pennisetum glaucum* and *P. alopecuroides*, infected with the fungus from rice, germinated on water agar and produced primary sporidia and both types of secondary sporidia essentially identical with those from rice that had been used in making the inoculations. Secondary sporidia from *P. glaucum* and *P. alopecuroides* were used separately to inoculate rice, but no infection was secured.

FIG. 1. Spores of *Neovossia barclayana:* A, B. from rice (median and surface views); C. from *Pennisetum glaucum*, artificially inoculated with the fungus from rice; D. from *P. alopecuroides*, artificially inoculated with the fungus from rice; E. from *P. orientale* var. *triflorum* (part of type). (All × 550.)

TABLE I

SIZE RANGES OF CHLAMYDOSPORES OF *Tilletia horrida*, *T. pennisetina*, AND *Neovossia barclayana* AS REPORTED BY VARIOUS INVESTIGATORS

Fungus	Original	Inoculated	Source	Spore diameter (μ)	Investigator
Tilletia horrida	*Oryza sativa*	—	Japan	18–26	Takahashi (**26**)
Tilletia horrida	*Oryza sativa*	—	Java	22–26	Rutgers (**22**)
Tilletia horrida	*Oryza sativa*	—	Philippines	22–31	Reyes (**21**)
Tilletia horrida	*Oryza sativa*	—	(General)	22–33	Clinton (**9**)
Tilletia horrida	*Oryza sativa*	—	S. Carolina	26–30	Anderson (**2**)
Tilletia horrida	*Oryza sativa*	—	S. Carolina	23–33	Writers
Tilletia horrida	*Oryza sativa*	—	Louisiana	22–28	Fulton (**13**)
Tilletia horrida	*Oryza sativa*	—	Louisiana	24–35	Writers
Tilletia horrida	*Oryza sativa*	—	Arkansas	26–35	Writers
Tilletia horrida	*Oryza sativa*	—	Texas	23–35	Writers
Tilletia horrida	*Oryza sativa*	*Pennisetum alopecuroides*	Texas	18–27	Writers
T. pennisetina	*Pennisetum alopecuroides*	*Pennisetum glaucum*	Texas	16–27	Writers
T. pennisetina	*Pennisetum setosum*	—	China	20–26	Sydow (**25**)
T. pennisetina	*Pennisetum setosum*	—	Korea	22–31	Writers
Neovossia barclayana	*Pennisetum orientale* var. *triflorum* (type)	—	Japan	19–35	Writers
Neovossia barclayana	*Pennisetum orientale* var. *triflorum* (part of type)	—	India	17–22	Brefeld (**5**)
			India	18–27	Writers

* From specimens communicated by Anderson from South Carolina in February 1899, and therefore presumably from the same source as his material (**1,2**). It should be added that in the "exchange list," published by Mrs. F. W. Patterson (**20**), specimen No. 204 is listed as "*Tilletia horrida* on *Oryza sativa*. Japan." The corresponding specimens in the Mycological Collections, Bureau of Plant Industry, give the collection date as "1898." In examining these specimens in connection with the present investigations, a revised label and special note, prepared by Mrs. Patterson, were found attached to a packet enclosing some of the above material. These state that the material was communicated by A. P. Anderson from South Carolina, February 24, 1899, and that the seed rice had been imported from Japan in 1896. It seems, therefore, that the source of the material referred to by Mrs. Patterson under her No. 204 and the corresponding specimens distributed to various herbaria should have "South Carolina" as the source locality rather than "Japan." The date of collection "1898" doubtless is correct, as that is when Anderson (**1,2**) reported the smut as having occurred near Georgetown, South Carolina.

It is clear from TABLE I that there is a considerable range in chlamydospore size for *Tilletia horrida* on rice and some apparent discrepancies between the measurements given by different investigators. These differences, however, are no doubt due to differences in making the measurements. For example, the measurements made by the writers include the scales on the spores and also the surrounding hyaline membrane. These were included because they are considered as parts of the spore. Reyes (**21**) states she did likewise. Clinton (**9**) apparently did also. Thus the measurements given by these authors are in essential agreement with those given by the writers. Those given by the other investigators referred to are smaller presumably because either one or both of the structures referred to may not have been included in their measurements. In any case, the range of chlamydospore size is so great that even several microns difference could scarcely be considered very significant. On the whole, therefore, the measurements of the chlamydospores from rice are considered in fair agreement.

It is especially noteworthy, however, that when *Tilletia horrida* from rice was grown on *Pennisetum glaucum* and *P. alopecuroides*, the chlamydospore size decreased from 23–35 μ on rice to 16–27 μ and 18–27 μ, respectively, on the latter two hosts. Furthermore, these measurements of spores from *P. glaucum* and *P. alopecuroides* are in essential agreement with those given by Sydow (**25**) for *Tilletia pennisetina* on *P. alopecuroides* from China and with those found by the writers for *Neovossia barclayana* (part of type) on *P. orientale* var. *triflorum* from India. The size-range of the chlamydospores on *P. setosum* apparently approaches that of the spores on rice.

As shown in FIG. 1, the spore-wall markings of *Tilletia horrida* on rice (A, B) are distinctly coarser than those of *N. barclayana* (type) (E), yet when *T. horrida* from rice was grown on *Pennisetum glaucum* (C), or on *P. alopecuroides* (D), the spore-wall markings were distinctly finer than those of the spores from rice (A, B). In fact, some of the spores from *P. glaucum* were found to have spore-wall markings identical with those of the spores from the type material of *N. barclayana*. The spore-wall markings of

the spores from *T. horrida* from rice grown on *P. alopecuroides* (FIG. 1, D) are intermediate between those of *T. horrida* on rice (FIG. 1, A, B) and those of the type material of *N. barclayana* and the rice fungus on *P. glaucum*. Both on *P. glaucum* and *P. alopecuroides*, however, there is a considerable range in the degree of fineness of spore-wall markings as shown in FIG. 1. There was, likewise, a considerable range in the depth of color of the spores from those two sources, the more mature spores being very dark and the immature ones being almost hyaline, the same as on rice.

In all of the material studied, namely, *Tilletia horrida* on rice and inoculated on *P. glaucum* and *P. alopecuroides; T. pennisetina* on *P. alopecuroides* and *P. setosum;* and *Neovossia barclayana* (type) on *P. orientale* var. *triflorum*, fragmentary appendages occasionally were noticeable particularly on the younger spores, indicating that the spores were borne singly and terminally, essentially as described for the type species of the genus *Neovossia, N. moliniae*. In all the cases studied, the appendages were distinctly less prominent than in *N. moliniae*. Because of the presence of the fragmentary appendages in the rice kernel smut fungus, however, and also because of the type of spore germination and the formation of dense heads of primary sporidia that do not fuse, and, further, because of the formation of two kinds of secondary sporidia, it seems clear that the rice kernel smut fungus really belongs in the genus *Neovossia*, rather than in the genus *Tilletia*. This conclusion is in agreement with that of Padwick and Azmatullah Khan (**18**).

Furthermore, since it has been found that the rice kernel smut fungus, *Tilletia horrida*, is able to infect certain species of *Pennisetum*, and that it produces on them spores that are essentially identical with those of *Neovossia barclayana* Brefeld originally described on *Pennisetum orientale* var. *triflorum* (*P. triflorum*), it seems advisable to accept the older name, *N. barclayana* Brefeld, for this smut on rice as well as for the smuts on the other species of *Pennisetum* referred to.

Saccardo and P. Sydow (**23**) transferred *N. barclayana* to the genus *Tilletia* apparently because Brefeld had not described appendages on the spores. As noted above, however, fragmentary appendages have been found on the spores from type material of this

species. Furthermore, the same authors (**24**) later accepted the genus *Neovossia* and placed their own combination, *Tilletia barclayana,* in synonymy under *N. barclayana* Brefeld. At the same time, they defined the genus *Neovossia* Koern. as *"Tilletiae affinis, differt sporidiolis numerosissimis in capitulum unitis"* and included three species: *N. moliniae* (Theum.) Koern., *N. barclayana* Brefeld, and *N. iowensis* Hume and Hodson.

The following emended description of the genus *Neovossia* is given to permit the inclusion of species with appendages fragmentary, or more or less lacking, provided that such species possess the other characters of the genus.

NEOVOSSIA Koern. emend.

Sori usually in ovaries, forming a somewhat dusty spore-mass; spores simple, produced singly in the swollen ends of special, fertile hyphae, which invest the spores and usually taper into elongate or fragmentary hyaline appendages. Some of these may persist even on mature spores. Spores germinate by promycelia, which produce numerous terminally-clustered, more or less filiform, primary sporidia. These germinate without conjugation and give rise to a mycelium that produces secondary sporidia of two kinds.
Type, *Vossia moliniae* Thuem.

The nomenclature, synonymy, description, host range, and type locality of the kernel smut fungus on rice and *Pennisetum* spp. are as follows:

NEOVOSSIA BARCLAYANA Brefeld, Untersuch. Gesamtgeb. Mykol. **12**: 170. 1895.

Tilletia horrida Tak. Bot. Mag. Tokyo **10** (2): 20. 1896.
Tilletia barclayana Sacc. and Syd.; Sacc. Syl. Fung. **14**: 422. 1899.
Tilletia pennisetina Syd. Ann. Myc. **27**: 421. 1929.
Neovossia horrida Padw. & A. Khan, Imp. Mycol. Inst. Mycol. Papers **10**: 2. 1944.

Sori partly or entirely destroying the ovaries; in *Oryza sativa* the sori usually remain almost entirely concealed by the enveloping glumes, but in *Pennisetum* spp. the sori protrude more or less; chlamydospores, each surrounded by smooth, hyaline membrane, showing different stages of development, the young spores being

thick-walled, hyaline, with wall, inside the hyaline envelope, at first smooth, and later, much roughened and each usually with a fragmentary appendage, a remnant of the hypha that produced it. As the spores mature, the walls take on color, at first light and then darker cinnamon brown, and later darker brown, many becoming almost opaque; spores spherical to subspherical with coarse (on *O. sativa*) or moderately coarse (on *Pennisetum* spp.) hyaline or slightly tinted, erect or slightly curved scales, which (especially on *O. sativa*) show, in lateral view, at the circumference of the spore as a jagged band 1–4 μ wide; in top view, as more or less irregular, pointed protuberances 1–4 μ across at their bases; occasionally the scales are elongated into more or less irregular ridges. On *Pennisetum* spp. these spore protuberances are similar to those on *O. sativa* except that usually they are distinctly finer. The scales or protuberances usually are enclosed by a smooth, hyaline membrane about 1 μ thick, which tends to hold them down, sometimes curving them to the right or left, or simply crumpling down their tips. In old material or on germinating spores, it is not especially uncommon to find occasional spores with the hyaline membrane broken or more or less absent. In such cases, the scales tend to be erect and therefore especially prominent. On the mature spores the appendages are fragmentary or lacking. Spores from *O. sativa* 18–26 × 26–35 μ; on *Pennisetum* spp., 16–22 × 22–35 μ.

On germination, the chlamydospores produce nonseptate promycelia, which bear at their simple or branched tips dense heads of 20–60 hyaline, filiform, non-septate, primary sporidia, 1.5–2 × 38–53 μ. When the promycelia are branched (**5**, **26**, **32**), a reduced number of primary sporidia are borne on the tip of each branch.

On water agar or potato dextrose agar, the primary sporidia produce branched hyphae on which two kinds of secondary sporidia are borne on short branches, usually singly, the one kind, obclavate-allantoid, non-septate, 2–3 × 10–14 μ, and the other kind, filiform to very narrowly obclavate, non-septate, usually slightly curved, 1.5–2 × 22–42 μ. The former are forcibly discharged, and after discharge, may enlarge to 4 × 16 μ, and germinate by sending out a germ-tube at each end. The filiform secondary sporidia apparently also are forcibly discharged, but less violently than the former, and germinate from their ends or sides.

On Poaceae:

Oryza sativa L., Arkansas, Louisiana, and Texas in the United States; also India (**6**), Burma, Siam, Malay Archipelago (**34**), Indo-China, China, Japan, Java, Philippine Islands.

Pennisetum alopecuroides (L.) Spreng., China (**28**, as *Tilletia pulcherrima* Ell. & Gall.; also from artificial inoculation).
P. glaucum (L.) R. Br., Senegal (**8**; also from artificial inoculation).
P. setosum (Swartz) L. Rich. (*P. purpurascens*), Japan, Korea.
P. orientale var. *triflorum* (Nees) Stapf (*P. triflorum*) (type), India (**5**, **7**).
Type locality: Simla, India on *Pennisetum orientale* var. *triflorum* (Nees) Stapf (*Pennisetum triflorum*).

DISCUSSION

The reported variation in spore size and marking in *Neovossia barclayana* after passage through the two species of *Pennisetum*, as described above, appears to be somewhat similar to the condition reported by Fisher (**12**) in *Ustilago bullata* Berk. He found a variation of about 4 μ in spore diameter in collections from various species of *Bromus* and also variations in epispore characters (**12**, p. 414) ranging from very minutely echinulate-verrucose to bluntly echinulate-verrucose. On species of *Agropyron* and *Hordeum*, there was less variation in spore size and epispore markings.

The writers found a difference of 2–3 μ in spore measurements on *Oryza sativa* and 6–8 μ on *Pennisetum* spp.

Numerous attempts to reproduce the disease on rice have been unsuccessful using naturally-infested seed and seed artificially inoculated either with chlamydospores from rice or with sporidia from chlamydospores from *Oryza sativa*, *Pennisetum glaucum*, or *P. alopecuroides*.

The reactions of the hosts also indicate that certain species of *Pennisetum* may be more susceptible to the fungus than rice, as the ovaries of these grasses are practically entirely destroyed and, in most cases, all the florets are attacked, while in rice only a part or all of the endosperm is destroyed and only 3 or 4 florets of a panicle are attacked. The latter may perhaps be due to the more open structure of the panicle as compared with the compact structure in the spikes of the species of *Pennisetum*. The fact that no infection was obtained in rice inoculated in various ways, and that it was obtained in *Pennisetum* spp., also indicates strongly that the attacked species of *Pennisetum* are more susceptible than *Oryza sativa*. It may be noted also that the report by Winkler (**34**) that

Tilletia horrida occurs on several wild grasses in Louisiana is due to erroneous identification (**1**, **23**) of the rice smut fungus as *T. corona* Scrib., which later was reported by Tracy and Earle (**29**) as occurring "on various grasses." Then when Earle (**11**) in 1899, and Anderson (**3**) in 1902, corrected the identification of the rice smut fungus to *T. horrida* Tak., Winkler (**34**) apparently considered the two fungi identical, which they are not. He, therefore, wrongly ascribed the host range of *T. corona* to *T. horrida*.

The status of *Tilletia ajrekari* Mundkur (**17**, p. 103) on *Pennisetum glaucum* L. Br. (= *P. typhoides* Stapf) in relation to *Neovossia barclayana*, as interpreted above, has not been fully determined. True, the chlamydospores of *T. ajrekari* are slightly smaller (14–21 μ) and the epispore protuberances somewhat coarser than those noted above for *N. barclayana*. However, in view of the fact that the size and markings of the spores of the rice smut fungus have been found to vary so widely in experimental material, and also since *T. ajrekari* is described on the identical host found to be susceptible to the rice smut fungus, and also since the rice smut fungus and also *N. barclayana*, as originally described, both occur in India (**5**, **6**), it seems very likely that *T. ajrekari* is but a variant of *N. barclayana* Brefeld, as here interpreted. It seems possible, therefore, that the lone sorus described by Mundkur (**17**) may have come from a chance infection from some other species of *Pennisetum* or possibly even from rice.

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AN UNDESCRIBED SPECIES OF ERGOT ON PANICUM MAXIMUM JACQ. VAR. COMMON GUINEA

T. THEIS [1]

(WITH 1 FIGURE)

During a recent survey of forage-crop diseases in Puerto Rico, it was observed that guinea grass (*Panicum maximum* Jacq. var. common guinea), an important forage grass, was frequently infected with ergot. The sphacelial stage of ergot on *P. maximum* has been reported by Shepherd (**5**) and Thomas *et al.* (**8**). Viegas (**9**) observed the ergot parasite *Cerebella* spp. on *P. maximum*, and this was also seen in Jamaica by Martyn (**3**). The sclerotial stage has been found in Puerto Rico. It is the purpose of this paper to describe this species, which is believed to be new.

MATERIALS AND METHODS

When artificial inoculations were made with ergot, the inflorescences were covered with parchment paper bags from the time previous to flowering until the experiment was finished. When the first flowers appeared, the inoculations were begun. Entire inflorescences were sprayed daily with a conidial suspension for a period of 2 weeks.

The conidial inoculum was prepared from 10 flowers that were exuding honeydew. These flowers were mascerated in 25 cc. of distilled water. The coarse particles were removed by filtration through two layers of cheesecloth. The filtrate was sprayed on the flowers with a De Vilbiss No. 15 atomizer, until the entire inflorescence was thoroughly wetted.

[1] The author expresses appreciation to Edith K. Cash for preparing the Latin description and to C. L. Lefebvre for counsel in connection with these studies.

There are discrepancies in the literature regarding *Panicum maximum* because of the presence of numerous variant forms of the grass. All references to guinea grass in this paper are based on the key devised by Warmke (**10**) and identified as *P. maximum* var. common guinea.

CROSS INOCULATION

In the autumn of 1949, sphacelial stages of ergot were observed on guinea grass and also on gamelotillo (*Paspalum plicatulum* Michx.). This situation appeared similar to that reported by Shepherd (**5**), who observed a conidial stage of ergot on *Paspalum dilatatum* Poir. and on *Panicum maximum*. Both of these grasses had been thought to be infected with *Claviceps paspali* Stev. & Hall by Sprague (**6**). Since the ergot on *Paspalum plicatulum* has been reported by Stevens (**7**) to be *C. paspali*, the possibility existed that the ergot of guinea grass might be identified by cross-inoculation tests with *P. plicatulum*.

Two experiments of cross-inoculation with the sphacelial stages from *Paspalum plicatulum* and *Panicum maximum* were conducted. In both experiments, four inflorescences of each species were inoculated with spores collected from the same species, four were inoculated with spores of the opposite species, and four were checks.

The sphacelial stage of ergot was recoveerd from the plants inoculated with spores collected from the same species, but the cross-inoculations and the checks were free of disease. From this it appears that the ergots on the two grasses are different species or different races of *Claviceps paspali*.

IDENTIFICATION

The sclerotial stage (FIG. 1, A) of guinea grass ergot was first observed in the arid southwestern region of Puerto Rico. The infection in this area was extensive and in November and December of 1949 and 1950 large quantities of sclerotia were collected. These sclerotia germinated (FIG. 1, B) when sown in shallow pans of soil, thus enabling identification to be made. The following is a description of ergot of guinea grass:

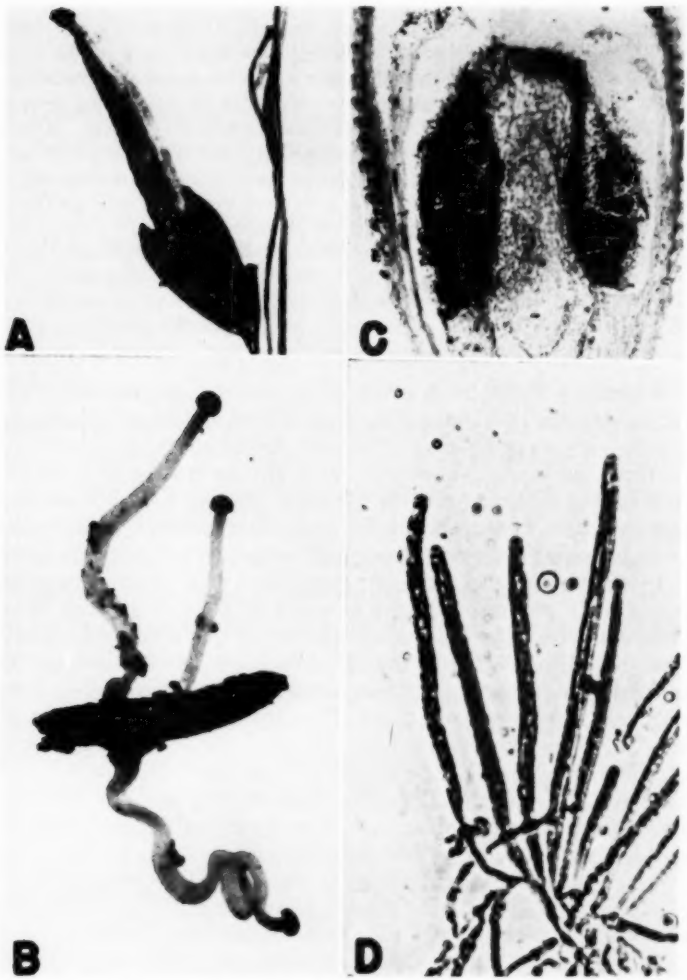

FIG. 1. *Claviceps maximensis*. A. Sclerotium showing adherent glumes and cap of dried hyphae of sphacelial stage, × 9; B. Stipes and capitula of germinated sclerotium, × 6; C. Convoluted hyphae of sphacelial stage on ovary of *Panicum maximum* var. common guinea, × 365; D. Asci showing caps on distal end, × 600.

Claviceps maximensis sp. nov.

Sclerotiis 2-9 mm. longis, 1 mm. latis, brunneis vel griseo-brunneis, intus albis, rectis usque subcurvatis vel tortis, supra attenuatis, apice hyphis aridis sphacelioidibus pileatis, longitudinaliter furcatis, ovaria hospitis restituentibus, glumis affixis restantibus; in diebus 20 germinantibus et stromata 1–5 gerentibus; stipitibus in longitudine variabilibus, 0.4–0.6 mm. in diam., glabris, pallide viridibus, in maturitate flavescentibus, flexuosis; capitulo 0.7–1.0 mm. in diam., subgloboso, flavidulo dein brunnescenti, colligationem cum stipite superpendente; superficie e peritheciis protrudentibus colliculosa; peritheciis lageniformibus, in capitulum immersis, per superficiem superiorem totam distributis, collis leniter protrudentibus, ovatis, 240–280 μ longis, 120–160 μ latis; ascis hyalinis, cylindricis, 2.5–3.0 × 105–139 μ, basim versus attenuatis, apice pileo hyalino praeditis, octosporis; paraphysibus carentibus; ascosporis hyalinis, filiformibus, 0.5 × 95–126 μ; conidiis hyalinis, ellipticis, eseptatis, 10–30 × 3.5–11.0 μ, ex hyphis convolutis in ovariis oriundis.

Sclerotia 2–9 mm. long, 1 mm. wide, brown to gray-brown, white inside, straight to slightly curved or twisted, tapering upward, tip usually with a cap of dried sphacelial-stage hyphae, longitudinally furrowed, replacing host ovaries, host glumes remaining attached; germinating in approximately 20 days, bearing 1 to 5 stromata; stipes variable in length, 0.4–0.6 mm. in diameter, smooth, pale-green becoming yellow as the capitulum matures, flexuous; capitulum 0.7 to 1.0 mm. in diameter, subglobose, light yellow becoming brown as it matures, overlapping point of attachment with stipe, surface uneven due to slightly projecting perithecia; perithecia flask-shaped, immersed in the capitulum and distributed over the entire upper surface, neck projecting slightly, ovate, 240–280 μ long and 120–160 μ wide; asci (FIG. 1, D) hyaline, cylindrical, 2.5–3.0 × 105–139 μ, tapered toward point of attachment, apex with a hyaline cap, 8-spored; no paraphyses; ascospores hyaline, filiform, 0.5 × 95–126 μ; conidia hyaline, elliptical, aseptate, 10–30 × 3.5–11.0 μ from convoluted hyphae (FIG. 1, C) in ovary of flower; chemical tests for ergot alkaloids negative.[2]

Found infecting inflorescences of *Panicum maximum* var. common guinea, by T. Theis at La Parguera, Puerto Rico, November 12, 1949. Type specimens have been deposited in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

[2] The Penick Pharmaceutical Company, New York, tested guinea grass ergot along with their commercial rye ergot samples in routine tests for alkaloids. The colorimetric method was employed, involving extraction of alkaloids and comparison with Ergotoxine Ethanesulfonate standard according to the procedure in the British Pharmacopoeia. They found that fresh guinea grass ergot exhibited none of the usual activities of ergot and considered it probable that none of the ergot alkaloids were present.

The ergot on guinea grass is macroscopically similar to *Claviceps uleana* P. Henn. reported on a *Panicum* species in Brazil (**1**). The latter fungus, however, is generally smaller and more delicate. There is a difference in ascus structure also. In *C. uleana*, the tip of the ascus is reported to be rounded. The ascus of *C. maximensis*, on the other hand, has a distinct hyaline cap. These caps appear to come free easily and expose the terminal ends of the ascospores. Such structures have been reported before by Moeller (**4**) who observed hyaline caps on the asci of *Claviceps balansioides* A. Moell.

ENVIRONMENTAL RELATIONS

The environment appears to play an important role in determining the stage of development of the disease. Though guinea grass is best suited to arid conditions because of its drought resistance, it is found to some extent throughout Puerto Rico. In areas of moderate rainfall (40 inches per annum) to high (100 inches and over), the ergot infection of guinea grass has been observed to be restricted to the sphacelial stage. It is in the arid southwestern coast region that guinea grass ergot forms sclerotia. Here the rainfall is less than 40 inches per year and is confined to a few months in the fall. It is during this time that the plants flower, the fungus infects, proceeds through the sphacelial stage, and produces sclerotia. Sclerotia of guinea grass ergot have also been seen on St. Croix, Virgin Islands, where the climate is very much like that of the southwestern part of Puerto Rico (**2**).

Ergot infection in the southwestern part of Puerto Rico is more severe than in other parts of the island. This is a dairy region, and large acreages are devoted to guinea grass pastures. For two consecutive years an examination of the fields showed that almost every floret in most inflorescences was infected. In spite of the presence of hyperparasites (*Cerebella andropogonis* Ces., and *Cladosporium* sp.) large numbers of sclerotia were formed. Of 20 inflorescences picked at random, an average of 52 sclerotia per inflorescence was found. Farmers of the southwestern region report seeing this disease for many years. Apparently environmental conditions in this area favor yearly epiphytotics of ergot on guinea grass.

SUMMARY

A new species of ergot found on guinea grass (*Panicum maximum* var. common guinea) in Puerto Rico has been described and named *Claviceps maximensis*.

Chemical tests indicate that none of the usual ergot alkaloids are present in the sclerotia.

The stage of development of this disease is determined by some environmental condition. In arid regions where plant growth is confined to few months of the year, sclerotia are formed. In more humid regions with a long growing period the infection is restricted to the sphacelial stage only.

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WESTERN FUNGI. III [1]

WM. BRIDGE COOKE [2] AND CHARLES GARDNER SHAW [3]

6. NEW AND INTERESTING SPECIES OF FUNGI IMPERFECTI

During the course of routine identifications of various fungi collected in Washington and in other western states, the following species were found to be new or of special interest. The species discussed are all members of the Fungi Imperfecti. In none of the specimens cited below was a mature perfect stage associated with the imperfect stage.

The writers wish to acknowledge the assistance of Miss Elizabeth C. Bridge, Winsor School, Boston, Massachusetts, in the preparation of the Latin diagnoses.

Phyllosticta maianthemi W. B. Cooke, sp. nov.

Maculis indefinitis, latis, nigrescentibus, deinde in totis foliis; pycnidiis hypophyllis, numerosis, nigris, 70–125 μ diam.; ostiolo poroso; intus conidiophoris, 5–6 × 1–2 μ, hyalinis, indutis; sporis hyalinis, cylindricis, bacillaribus, 2.5–3.5 × 1 μ.

Fruiting on older leaves between the veins and eventually blackening the entire blade, younger leaves apparently uninfected; pycnidia hypophyllous, mostly crowded, black, immersed in leaf tissue, 70–125 μ in diameter, ostiole appearing as a surface pore; conidiophores lining the pycnidium, 5–6 × 1–2 μ, hyaline; spores hyaline, cylindric, bacillar, 2.5–3.5 × 1 μ.

Host: *Maianthemum dilatatum* (Wood) Abrams.

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TYPE and specimen examined: WASHINGTON: Jefferson Co.: Kalaloch, 30 miles south of Bogachiel, along U. S. Highway 101 in Sitka spruce woodland, Aug. 25, 1950. Collected by W. B. & V. G. Cooke (*26872*) and R. Binegar (WSC-PP *30122*).[4]

A visit to the type locality a year later failed to reveal any additional material of this species.

Phyllosticta hosackiae W. B. Cooke, sp. nov.

Maculis in toto foliolo; pycnidiis hypophyllis, numerosis, nigris, 75–100 μ diam.; intus sporophoris, 5–10 $\times$ 1 μ, indutis; sporis hyalinis, bacillaribus, 3–4 $\times$ 0.7–1 μ.

Spots covering the entire leaflet, containing large numbers of black pycnidia 75–100 μ in diameter, pycnidia hypophyllous, rather thin-walled, lined with conidiophores 5–10 $\times$ 1 μ; pycnidiospores hyaline, bacillar, 3–4 $\times$ 0.7 μ.

Host: *Lotus douglasii* Greene.

TYPE and specimen examined: WASHINGTON: Klickitat Co.: Falcon Valley, Sept. 11, 1889. Collected by W. N. Suksdorf (*149*) (WSC-PP *27765*).

Phyllosticta erythronii sp. nov.

Maculis primo minutis, deinde grandis, sparsis vel aggregatis, brunneis; pycnidiis amphigenis, sparsis vel congregatis, atro-punctatis, brunneis, ostiolatis, 60–125 μ diam., subglobosis; sporis hyalinis, cylindraceis, bacillaribus, 3–3.5 $\times$ 0.5–1 μ.

Fruiting on old, dying leaves, forming small to large, isolated to confluent spots browning the leaf tissue; pycnidia amphigenous, scattered to gregarious, black in mass, punctate, without asteroid hyphae in surrounding tissue; pycnidia brown, thin-walled, ostiolate, 60–125 μ in diameter, subglobose, ostiole raised above the surface of the leaf; pycnidia appear to be formed of interwoven hyphae, 3–4 μ in diameter, nearly hyaline below, extended above into a series of blackened threads which form the margin of the ostiole; spores hyaline, cylindric, bacillar, 3–3.5 $\times$ 0.5–1 μ.

Host: *Erythronium parviflorum* (S. Wats.) Good.

TYPE and specimen examined: WYOMING: Carbon Co.: Mirrow Lake, Medicine Bow National Forest, August 9, 1950. Collected by C. G. Shaw, Mycological Society Foray (WSC-PP *30134*).

[4] Specimens bearing "WSC–PP" accession numbers are deposited in the Mycological Herbarium, Department of Plant Pathology, The State College of Washington.

Phyllosticta eriogoni W. B. Cooke, sp. nov.

Maculis irregularibus, 2 mm. diam., vel 1.5 × 0.5 cm., brunneis, amphigenis, luteo-marginatis; pycnidiis amphigenis, sparsis, 125–175 μ diam.; intus conidiophoris hyalinis, 8–12 × 5–7 μ, indutis; sporis hyalinis, globosis vel ovoideis, lenibus, 6–8 μ diam. vel 6–8 × 7–10 μ.

Spots of irregular size, 2 mm. in diameter to 1.5 × 0.5 cm., brown, amphigenous, with a yellow halo; pycnidia scattered, 125–175 μ in diameter, immersed in the tissue of the spot, with marginal cells slightly darker than those of the leaf; conidiophores short, hyaline, 8–12 × 5–7 μ, forming a palisade inside the pycnidium; pycnidiospores hyaline, spherical to ovate, smooth, crowded, 6–8 μ in diameter to 6–8 × 7–10 μ.

Host: *Eriogonum elatum* Dougl.

TYPE and specimen examined: WASHINGTON: Klickitat Co.: Viola, near Bingen, June 2, 1926. Collected by W. N. Suksdorf (*1137*) (WSC-PP *28817*).

Phoma eriogoni W. B. Cooke, sp. nov.

Pycnidiis nigris, caulicolis, 450 μ diam.; ostiolis papillatis; contextu crasso, atro-brunneo, pseudoparenchymatico, cellulis 3–5 μ diam.; intus sporophoris hyalinis, 8–10 × 3–4 μ, indutis; sporis hyalinis, cylindricis, rectis vel subcurvulis, 3–4 × 1–1.5 μ.

Pycnidia black, thick-walled, 450 μ in diameter, seated on the surface of dead stems or root-stocks, papillate ostiolate; wall formed of several layers of black-brown, pseudoparenchymatous cells 3–5 μ in diameter, compact; conidiophores lining cavity of pycnidium, 8–10 × 3–4 μ, hyaline; spores hyaline, cylindric, slightly curved, 3–4 × 1–1.5 μ.

Host: *Eriogonum marifolium* T. & G.

TYPE and specimen examined: CALIFORNIA: Siskiyou Co.: Mt. Shasta, South Gate, 8000 ft., Aug. 24, 1949. Collected by W. B. & V. G. Cooke (*25630*) (WSC-PP *30133*).

This species is similar to *P. herbarum* Westd. but the spores are smaller.

Macrophoma chrysothamni W. B. Cooke, sp. nov.

Maculis amphigenis, in toto folio; pycnidiis nigris, 150–220 μ diam., tenuisculis; ostiolo brevissime papillato; contextu pseudoparenchymatico; cellulis angulatis, atrobrunneis, 3.5–7 μ diam.; intus conidiophoris 15–18 × 5–6 μ, dense indutis; conidiis hyalinis, ovoideis vel cylindraceis vel subfusiformibus, 18–25 × 7–8.5 μ.

Pycnidia developing throughout the leaf but only on occasional leaves, black, thin-walled, 150–220 μ in diameter, ostiolate, the ostiole usually appearing as a slightly raised papilla; pycnidial wall composed of a layer of brown to blackish-brown, angular pseudoparenchymatous cells 3.5–7 μ in diameter, containing a densely compact palisade of conidiophores 15–18 × 5–6 μ; conidia hyaline, 1-celled, 18–25 × 7–8.5 μ, ovate to cylindric to tear-shaped and slightly constricted near the base, usually with a basal papilla.

Host: *Chrysothamnus nauseosus* (Pall.) Britt. var. *occidentalis* (Greene) Peck.

TYPE and specimen examined: CALIFORNIA: Siskiyou Co.: North side of Mt. Shasta above Bolam Station in the chaparral, 5000 ft., Aug. 26, 1949. Collected by W. B. & V. G. Cooke (*25671-A*) (WSC-PP *30117*).

Sphaeropsis vaccinii sp. nov.

Maculis amphigenis, brunneis, supra marginis carneo-purpurascentibus; pycnidiis epiphyllis, globosis, 150–300 μ diam., foliis immersis; contextu crasso, nigro, pseudoparenchymatico; ostiolis minutis; intus conidiophoris, 5 × 5 μ indutis; conidiis lutescentibus, ovoideis vel subfusiformibus, 11–13 × 6–7.5 μ.

Spots brown, surrounded by reddish-purple margins above; pycnidia epiphyllous, globose, 150–300 μ in diameter, with thick walls formed of black pseudoparenchymatous tissue, ostiole minute; conidiophores short, 5 × 5 μ, lining the pycnidium; conidia yellowish, ovate to pyriform, abundant, 11–13 × 6–7.5 μ.

Host: *Vaccinium ovatum* Pursh.

TYPE and specimen examined: WASHINGTON: Pacific Co.: Cranberry-Blueberry Experiment Station, Long Beach, July 10, 1948. Collected by J. P. Meiners and G. W. Fischer (WSC-PP *30120*).

Cytospora eriogoni W. B. Cooke, sp. nov.

Stromatibus sparsis, superficialibus vel erumpentibus, applanato-verrucosis, 250–500 μ diam., 2–20 locularibus; contexto superno e cellulis nigris, sclerotici, irregularibus, pseudoparenchymaticis; ostiolis punctiformibus; nitus sporophoris hyalinis, simplicibus vel bifurcatis, palisadibus, 10–25 × 2–3 μ, indutis; sporis hyalinis, rectis vel suballantoideis, (8–)10–11 × 3–4 μ.

Stromata black, superficial or erumpent, warted on the surface from the 2–20 locules, up to 0.5 mm. in diameter, surface formed of a layer of black or olive-black, sclerotized, irregular, pseudoparenchymatous cells; spores extruded without order through minute ostioles; conidiophores hyaline, 10–25 μ long, 2–3 μ in

diameter, sometimes bifurcate, in a dense palisade, arising from a compact tissue formed by dichotomous branching of hyphae from the base of the locule; conidia in elongated chains, 1-celled, hyaline, straight to slightly suballantoid, (8–)10–11 × 3–4 μ.

Host: *Eriogonum pyrolaefolium* Hook.

TYPE and specimen examined: CALIFORNIA: Siskiyou Co.: Mt. Shasta, South Ridge above Horse Camp, 9000 ft., Aug. 1947. Collected by W. B. & V. G. Cooke (*25631*).

Ascochyta caricis sp. nov.

Maculis amphigenis, pallidis, circularibus vel etiam irregularibus, deinde brunneis; pycnidiis atro-brunneis, subepidermicis, globosis vel subglobosis, 75–100 μ diam.; ostiolis indistinctis; intus sporophoris minutis, 4–6 × 2–3 μ indutus; sporis cylindricis, rectis vel curvulis, 1-septatis, hyalinis, non constrictis, 20–28 × 3–4 μ.

Infected part of leaf brown, spots pallid, dotted with the blackish brown, punctate pycnidia; pycnidia buried in the leaf tissue, ostiole present but indistinct, globose to subglobose, 75–100 μ in diameter; sporophores small, lining the pycnidium, 4–6 × 2–3 μ; spores cylindric, straight to curved, 1-septate, hyaline, not constricted at the septum, 20–28 × 3–4 μ.

Host: *Carex* sp.

TYPE and specimen examined: CALIFORNIA: Modoc Co.: 5 miles south of Pine Creek, July 28, 1950. Collected by R. Sprague (WSC-PP *30131*).

Ascochyta rhamni sp. nov.

Maculis circularibus vel elongatis, sparsis vel confluentibus, brunneis, 0.5–2 cm. diam., centris griseis; pycnidiis amphigenis, sparsis vel gregariis, globosis, contextu membranaceo, 100–300 μ diam.; ostiolo papillato; intus conidiophoris hyalinis, 5–10 × 3–4 μ, indutis; sporis hyalinis, 1-septatis, cylindraceis, rectis, 10–12 × 3–4 μ.

Spots round to ovate, scattered, brown, eventually becoming confluent, 0.5–2 cm. in diameter, with gray center; pycnidia amphigenous, scattered to gregarious, round, ostiolate, thin-walled, 100–300 μ in diameter, ostiole papillate; conidiophores lining pycnidia, 5–10 × 3–4 μ, hyaline; spores hyaline, 1-septate at maturity, cylindric, straight, 10–12 × 3–4 μ.

Host: *Rhamnus purshiana* DC.

TYPE and specimen examined: IDAHO: Shoshone Co.: Avery Creek, Sec. 17, R. 4 E., T. 22 N., Aug. 22, 1948. Collected by W. D. Yerkes, Jr. (WSC-PP *30123*).

Diplodina tridentatae sp. nov.

Maculis in toto folio, amphigenis, griseis; pycnidiis nigris, 150–400 μ diam., deinde cupulatis; contextu membranaceo, pseudoparenchymatico; intus conidiophoris atrobrunneis, 3–10 × 6–7 μ, indutis; conidiis seriatis, hyalinis, 1-septatis, 32–40 × 6.5–7.5 μ.

Infected leaves occasional, those infected covered with black, punctate pycnidia; pycnidia amphigenous, 150–400 μ in diameter, top half soon dehiscent, then resembling acervuli sunken in tissue and filled with spores, cup portion discrete, outlined in black with rather thin layer of dark brown pseudoparenchymatous cells; condiophores lining cup, 3–10 × 6–7 μ, concolorous with wall cells; spores seriate if not in chains, hyaline, 32–40 × 6.5–7.5 μ, 1-septate, lower cell slightly smaller.

Host: *Artemisia tridentata* Nutt.

TYPE and specimen examined: UTAH: Diamond Creek, Palmyra Forest Camp, Uintah National Forest, June 8, 1947. Collected by R. Sprague (WSC-PP *30125*).

Diplodina artemisiae Bres. apud Jaap is reported to have spores 8–10 × 2–2.5 μ. This is the only other species of this genus reported on a species of *Artemisia*.

Diplodina silenes W. B. Cooke, sp. nov.

Pycnidiis immersis, sparsis vel aggregatis, subcuticularibus, tectis in caulibus circumdantibus atro-punctatis, deinde cupulatis; epidermide caulium internodorum lutescenti; contextu pycnidiorum membranaceo hyalino, e cellulis radiantibus, 5–10 μ diam.; intus sporophoris hyalinis, 5–10 × 5 μ, indutis; sporis hyalinis, 1-septatis, non constrictis, 18–25 × 5–7 μ.

Caulicolous, not in spots but covering entire internodes for one-fourth to three-fourths of the stem, yellowish; pycnidia punctate, black, abundant, 60–150 μ in diameter; pycnidial wall 1–2 cells thick, cells 5–10 μ in diameter, inner layer hyaline, outer layer composed of dark cells arranged parallelly, radiating from the top; ostiole almost completely closed in mature pycnidia; conidiophores small, hyaline, 5–10 × 5 μ, forming a palisade around inside wall of pycnidium; spores hyaline, 18–25 × 5–7 μ, 1-septate, rarely 2-septate, lower cell sometimes smaller but septum usually central, spore wall not constricted at septum.

Host: *Silene douglasii* Hook. var. *douglasii*.

TYPE and specimen examined: CALIFORNIA: Siskiyou Co.: Mt. Shasta, north side above Bolam Station, Aug. 1949. Collected by W. B. & V. G. Cooke (*25660-B*), (WSC-PP *30132*).

Diplodina is differentiated from *Ascochyta* on the basis that the former occurs on stems and the latter on leaves. Monographic revision would be necessary to bring all species of *Diplodina* and *Ascochyta* described into line with this differentiation. Three species of *Diplodina* and *Ascochyta* have been reported previously on caryophyllaceous hosts: *Ascochyta dianthi* (A. & S.) Lib. with spores 14–16 × 3–4.5 μ has been reported on *Dianthus barbatus;* *Ascochyta silenes* Ell. & Ev. with spores 10–14 × 2.5–3 μ has been reported on *Silene antirrhina;* and *Diplodina passerinii* All. has been reported on *Antirrhinum* stems. The latter species has spores 7–12 × 2.5–3 μ. The spores of *Diplodina silenes* are much larger than those of the species previously described on members of the Caryophyllaceae.

Septoria scorzonellae sp. nov.

Maculis in toto folio, atro-griseis; pycnidiis 50–150 μ diam., nigris, contextu membranaceo; intus conidiophoris, 8–10 × 2 μ indutis; conidiis 18–46 × 1–2 μ, 0–2-septatis, hyalinis, rectis vel curvulis.

Pycnidia gregarious, 50–150 μ in diameter, black, rather thin-walled, covering leaves and scapes and coloring them gray-black; conidiophores arising from a basal hyaline tissue 10–15 μ thick, conidiophores 8–10 × 2 μ; conidia 18–46 × 1–2 μ, 0–2-septate, hyaline, straight to curved.

Host: *Scorzonella nutans* Geyer. [*Microseris nutans* (Geyer) Schul.-Bip. var. *major* (Gray) Nels. & Macbr. and *Ptilocalais nutans* (Geyer) Greene are considered synonyms.]

TYPE and specimen examined: WASHINGTON: Garfield Co.: Bear Creek, Hunter Spring Trail, Umatilla National Forest, July 11, 1948. Collected by C. G. Shaw, W. D. Yerkes, Jr., and R. Sprague (WSC-PP *30139*).

Septoria tellimae sp. nov.

Maculis in toto folio, nigris; pycnidiis nigris, sparsis vel gregariis, 60–100 μ diam.; ostiolis papillatis; conidiis filiformibus, hyalinis, 1–2-septatis, rectis vel curvulis, (36–)46–60 × 2–3 μ.

Pycnidia black, blackening the leaf, scattered to gregarious, papillate-ostiolate, 60–100 μ in diameter; conidia filiform, hyaline, 1–2-septate, straight to curved, (36-)46–60 × 2–3 μ.

Host: *Tellima parviflora* Hook.

TYPE and specimen examined: WASHINGTON: Whitman Co.: Wawawai, April 3, 1926. Collected by E. E. Honey (WSC-PP *20334*).

Three species of *Septoria* have been reported previously on related hosts. *S. saxifragae* Pass. has spores which measure 17–30 × 3 μ, while the spores of *S. mitellae* Ell. & Ev. measure 20–25 × 1 μ. These are below the range found in the present species. *S. albicans* Ell. & Ev. has small pycnidia, 45–50 μ in diameter, and spores measuring 50–75 × 1.5 μ. This fungus was found on species of *Saxifraga* and of *Heuchera*. It is considered distinct from the material described above on the basis of the larger pycnidia and the shorter and broader spores in the present material.

Ovularia lupini sp. nov.

Maculis primo brunneis, 2–3 mm. diam., deinde in toto foliolo; conidiophoris fasciculatis compactis, hyphophyllis, 100–150 μ diam., conidiophoris hyalinis, simplicibus, continuis vel septatis, flexuosis, nodosis, 72–108 × 3–5 μ; sporis hyalinis, ovatis vel globosis, non apiculatis, lenibus, continuis, 18–29 × 11–18 μ.

Spots starting as brown areas 2–3 mm. in diameter, finally taking over entire leaflets, covered by the greyish epidermis; conidiophores occurring in compact clusters 100–150 μ in diameter, conidiophores hypophyllous, hyaline, tortuous, geniculate, not septate in spore-producing area, 72–108 × 3–5 μ, spores borne on bumps on the conidiophores; spores hyaline, 1-celled, wall 0.5 μ thick, ovate to spherical, not apiculate, smooth, 18–29 × 11–18 μ.

Host: *Lupinus* sp.

TYPE and specimen examined: COLORADO: Near Skyline, Aug. 5, 1948. Collected by R. Sprague (WSC-PP *30141*).

Phyllosticta ferax Ell. & Ev. is present in the collection, but there appears to be no connection between the two species.

In the various host indices consulted, the species closest to this are *Ramularia lupini* J. J. Davis, occurring on *Lupinus* and possessing 1–3-septate spores 25–55 × 4–6 μ; and *Ovularia sphaeroidea* Sacc., occurring on *Lotus* sp., and possessing spores 8–10 μ in diameter or 8 × 9 μ.

Ramularia glauca Ell. & Ev., Jour. Myc. **9**: *222*. 1903.

Spots angular, with dark margins and tan to grey centers, up to 1 cm. in diameter; conidiophores arising in loose fascicles at the stomata; spores 18–21 × 3.5 μ, 1-celled, cylindric, hyaline.

Host: *Sambucus melanocarpa* Gray.

Specimen examined: IDAHO: Latah Co.: Sec. 17, R. 4 W., T. 40 N., July 22, 1948. Collected by C. G. Shaw and A. C. Goheen (WSC-PP *30138*).

This material fits Ellis and Everhart's description of *R. glauca* (spores 15–30 × 3–5 μ) better than that of *R. sambucina* Sacc., in which Peck's material is described as having spores 20–35 × 5–7 μ.

Ramularia lathyri sp. nov.

Maculis lutescentibus vel brunneis, nervis foliorum limitatis, deinde confluentibus, et in toto foliolo; conidiophoris fasciculatis, subflexuosis, ex ostiolo erumpentibus; sporis hyalinis, cylindraceis vel fusiformibus, continuis, 5–15 × 3.5–5 μ.

Spots brown to yellow, between the veins, becoming confluent, from 0.5 cm. to length of leaflet (5 cm.), 2–4 mm. wide; conidiophores arising in loose clusters from stomata; spores hyaline, 1-celled, cylindric with pointed ends to fusiform, 5–15 × 3.5–5 μ.

Host: *Lathyrus latifolius* L.

TYPE and specimen examined: WASHINGTON: Whitman Co.: Southwest end of Stadium, The State College of Washington Campus, Pullman, Aug. 2, 1948. Collected by C. G. Shaw (WSC-PP *30118*).

It is interesting to note that none of the host indices consulted lists a species of *Ramularia* on *Vicia, Lathyrus,* or *Pisum* species.

Ramularia orogeniae sp. nov.

Maculis nigris, sparsis vel aggregatis in toto folio; pustulatis amphigenis; conidiophoris pallide lutescentibus, fasciculatis, 20–25 × 5–6 μ, continuis; sporis hyalinis vel pallide lutescentibus, continuis, terminalibus, 36–45 × 4–6 μ; spermagoniis amphigenis, ostiolatis, atris, contextu pseudoparenchymatico; spermatiis hyalinis, rectis vel allantoideis, bacillaribus, 2.5–4 × 0.5–1 μ.

Spots at first scattered, then covering the whole leaf, leaf blackened except where superficial sporulation gives a flesh-colored bloom on the surface; stromata produced in the epidermis, later producing spermogonia above which the *Ramularia* conidiophores continue to produce conidia; conidiophores amphigenous, pale yellowish, fasciculate, 20–25 × 5–6 μ, aseptate; conidia single, terminal, hyaline to pale yellowish, 36–45 × 4–6 μ; spermogonia 50–75 μ in diameter, ostiolate, black, formed of one or two layers of pseudo-

parenchymatous cells; spermatia hyaline, straight to allantoid, bacillar, abundant, 2.5–4 × 0.5–1 μ.

Host: *Orogenia linearifolia* Wats.

TYPE and specimen examined: WASHINGTON: Garfield Co.: Teal Springs Meadow, Sec. 8, R. 42 E., T. 8 N., June 8, 1949. Collected by C. G. Shaw and W. D. Yerkes, Jr. (WSC-PP *30116*).

The close association of these two stages leaves little doubt but that they are the same species. If found independently, the spermagonial stage might be referred to *Phyllosticta*. There was no indication of the perfect stage.

Ramularia polemoniae sp. nov.

Maculis orbicularibus, 2–4 mm. diam., brunneis; conidiophoris geniculatis, hyalinis, fasciculatis, ex ostiolo erumpentibus, 5–25 × 4–5 μ, continuis vel septatis; sporis hyalinis, continuis, catenulatis, rectis vel subcurvulis, 15–36 × 2.5–4 μ.

Spots orbicular, 2–4 mm. in diameter, brown, in brown discolored portions of leaf; conidiophores geniculate, hyaline, arising in clusters of 2–10 or more from the stomata, 5–25 × 4–5 μ, 0–2-septate; spores hyaline, 1-celled, catenulate, straight to slightly curved, 15–36 × 2.5–4 μ.

Host: *Polemonium californicum* Eastwood.

TYPE and specimen examined: WASHINGTON: Garfield Co.: Bear Creek Camp, Umatilla National Forest, SW ¼, Sec. 16, R. 42 E., T. 8 N., July 11, 1948. Collected by R. Sprague, C. G. Shaw, and W. D. Yerkes, Jr. (WSC-PP *30124*).

Ramularia synthyridis W. B. Cooke, sp. nov.

Maculis determinatis, zonis purpureo-brunneis marginatis, centro albido; conidiophoris fasciculatis, sparsis, ex ostiolo erumpentibus, hyalinis, septatis; sporis hyalinis, 0–2-septatis, lenibus, 10–22 × 2.5–4 μ.

Spots well-marked, outlined by a broad, purple-brown border, with a white center; conidiophores fasciculate from the stomata, scattered, hyaline, septate; spores hyaline, 0–2-septate, smooth, 10–22 × 2.5–4 μ.

Host: *Synthyris reniformis* Benth.

TYPE and specimen examined: WASHINGTON: Skamania Co.: Dog Creek, Sept. 2, 1926. Collected by W. N. Suksdorf (*1151*) (WSC-PP *29383*).

Ramularia trillii sp. nov.

Maculis ovatis, deinde confluentibus, marginibus brunneis, centro griseo; conidiophoris sparsis, ex ostiolo stomatum erumpentibus, 10–15 × 2–3.5 μ; sporis hyalinis, continuis vel 1-septatis, 7–18 × 2.5–3.5 μ.

Spots ovate, becoming confluent, with brown borders and gray centers; conidiophores scattered, arising from stomata, 10–15 × 2–3.5 μ; spores hyaline, usually 1-celled, sometimes 1-septate, 7–18 (mostly 12–15) × 2.5–3.5 μ.

Host: *Trillium ovatum* Pursh.

TYPE and specimen examined: IDAHO: Latah Co.: Thatuna Hills, Moscow Mts., July 22, 1948. Collected by C. G. Shaw and A. C. Goheen (WSC-PP *30128*).

Didymaria anethiana W. B. Cooke, sp. nov.

Maculis nigris, orbicularibus vel ovoideis; conidiophoris septatis, denticulatis; sporis hyalinis vel lutescentibus, fusiformibus, 28–36 × 6–8 μ.

Spots black, orbicular to ovate, covering entire leaf segments, conidiophores breaking through stomata or epidermal cells and forming blackened pustules; conidiophores 3–5-celled, pointed at each cell where a spore is produced; conidia fusiform, 2-celled, hyaline to yellowish, 28–36 × 6–8 μ.

Host: *Anethum graveolens* L.

TYPE and specimen examined: OREGON: Near Lebanon, Linn Co., July 24, 1949. Collected by Watson and Randall (WSC-PP *30126*).

Ramularia agoseridis Ell. & Ev., Torr. Bot. Cl. Bull. **27**: 576. 1900.

Spots marginal, terminal or in the leaf blade, becoming confluent; conidiophores amphigenous, hyaline, 10–20 × 3–5 μ, fasciculate from the stomata and forming orbicular pustules 50–100 μ in diameter; producing spores serially or singly; spores hyaline, 1-celled, 18–25 × 3–4 μ, bluntly pointed.

Ramularia agoseridis has been found on *Agoseris grandiflora* (Nutt.) Greene in Idaho (WSC-PP *30129*) and Washington (WSC-PP *10274*) and on *A. heterophylla* (Nutt.) Greene (WSC-PP *17315*) and *A. laciniata* (Nutt.) Greene (WSC-PP *21524*) in Washington. In our material the conidiophores are hyaline in mass, while in the material distributed by Ellis & Everhart (Fungi Columbiani *452*) they are tinted light yellowish in mass, perhaps

indicating greater maturity in the latter specimen. Microscopically there is close agreement among all these specimens.

Gloeosporium arctostaphyli sp. nov.

Maculis primo purpureis, deinde nigris, marginibus purpureis; conidiophoris pustulatis, parallele instructis, ramosis, 10–25 × 2–3 μ; sporis hyalinis, continuis, cylindraceis, rectis, 3.5–10 × 1–3 μ.

Spots at first purple, then black with purple margins, more distinct from the upper surface; upper surface punctate with pycnidial or perithecial initials which form a stroma beneath the acervuli; conidiophores hyaline, branched, 10–25 × 2–3 μ; spores hyaline, 1-celled, 3.5–10 × 1–3 μ.

Host: *Arctostaphylos uva-ursi* (L.) Spreng. var. *coactilis* Fern. & Macbr.

TYPE and specimen examined: IDAHO: Bonner Co.: One-half mile south of Beaver Creek Ranger Station, June 18, 1948. Collected by R. Sprague and C. G. Shaw (WSC-PP *30140*).

Gloeosporium iliamnae sp. nov.

Maculis rotundatis, marginibus brunneis, centro griseo, 0.5–1 cm. diam; acervulis sparsis, epiphyllis, 60–80 μ diam., conidiophoris fasciculatis, parallele instructis, subdenticulatis, 26–36 × 5–8 μ; sporis hyalinis, curvulis, fusiformibus, 10.5–22 × 3–4 μ.

Spots round, brown-margined, gray to tan centered, 0.5–1 cm. in diameter; acervuli 60–80 μ in diameter, epiphyllous, one per lesion, conidiophores stout, irregular, 26–36 × 5–8 μ, sometimes somewhat geniculate, fasciculate; spores hyaline, non-septate, lunate to falcate, pointed at both ends, fusiform, 10.5–22 × 3–4 μ.

Host: *Iliamna rivularis* (Dougl.) Greene [*Sphaeralcea rivularis* (Dougl.) Torr.].

TYPE and specimen examined: OREGON: Harney Co.: Pine Canyon, Malheur National Forest, June 25, 1948. Collected by R. Sprague (WSC-PP *30136*).

Specimens, including the type, of *Ramularia sidalceae* Ell. and Ev., were lent by D. P. Rogers of the New York Botanical Garden. Our material described above differs from the *Ramularia* although the lesions are somewhat similar. In *R. sidalceae* the spores are produced on numerous small clusters of amphigenous conidiophores, the spores are 0–1-septate, essentially straight, apically blunt or rounded, and measure 12–22 × 2.5–3 μ. Speci-

mens of *R. sidalceae* studied included the type on *Sidalcea* sp. from British Columbia; a specimen on *Sidalcea* sp. from Corvallis, Oregon; one on *Sidalcea candida* A. Gray from Cummins, Wyoming; and one on *Sidalcea neo-mexicana* A. Gray from Sulphur Springs, Colorado. All these specimens, as well as one collected on *Iliamna rivularis* at Beech Creek Forest Camp, Oregon, June 25, 1947 (WSC-PP *30135*), agreed with the type of *Ramularia sidalceae* and are distinct from *Gloeosporium iliamnae*.

Cylindrosporium burkii sp. nov.

Maculis marginibus nigris vel atro-brunneis, centro albido vel griseo, sparsis, 0.5 cm. diam., vel 1 cm. diam., vel confluentibus, 3 cm. longis; acervulis epiphyllis, in maculis arum centro, 100–150 μ diam., numerosis; sporis 1–2-septatis, cylindraceis, rectis vel curvulis, 43–54 × 3–4 μ.

Spots with black or purple-black borders, white to ashy centers, 0.5 cm. in diameter, becoming 1 cm. in diameter and becoming confluent up to 3 cm. long; acervuli epiphyllous, abundant in centers of spots; 100–150 μ in diameter; spores 43–54 × 3–4 μ, 2–3-celled, cylindric, straight to curved, with rounded ends.

Host: *Lupinus burkii* Wats.

TYPE and specimen examined: IDAHO: Bonner Co.: Beaver Creek Ranger Station, Kaniksu National Forest, June 18, 1948. Collected by R. Sprague and C. G. Shaw (WSC-PP *30126*).

Earlier reports of *Cylindrosporium* spp. on *Lupinus* include *C. lupini* Ell. & Ev. with spores 15–23 × 1.25–1.5 μ, and *C. longisporum* Ell. & Dearn. with spores 60–105 × 3–4.5 μ. The length of the spores in the Idaho collection is intermediate between those of the two previously described species.

Cylindrosporium fraserae sp. nov.

Maculis atrobrunneis, centro griseo, circularibus; acervulis epiphyllis, sparsis, 30–75 μ diam.; sporis hyalinis, 1–5-septatis, rectis vel curvulis, 50–108 × 2–3 μ.

Spots delimited by a dark line, inside grayish to blackish, with a brownish halo on the outside; acervuli on the upper surface, scattered in the spots, 30–75 μ in diameter; spores hyaline, 1–5-septate, straight to curved, 50–108 × 2–3 μ.

Host: *Frasera macrophylla* Greene.

TYPE and specimen examined: COLORADO: Mesa Lake, Aug. 6, 1948. Collected by R. Sprague (WSC-PP *30137*).

Helminthosporium abietis sp. nov.

Longe lateque effusum, velutinum, nigrum, in caulibus et in foliis; subiculo adpresso ex hyphis olivaceis vel atro-olivaceis; hyphis septatis regularibus, constrictis, cellularis 8–10 μ diam.; hyphis irregulariter ramosis, anastomosantibus; conidophoris non differentiatis; sporis fusiformibus, 126–150 × 12–16 μ, 14–17-septatis, olivaceis, crassis, symmetricis.

Forming black mold-like patches on twigs and extending to include the older needles still attached to twigs, formed of loosely interwoven olive-green to blackish olive-green hyphae with isodiametric cells constricted at septa, 8–10 μ in diameter; hyphae irregularly branched, occasionally anastomosing with H-pieces; conidia produced at occasional points, fusiform, 126–150 × 12–16 μ, 13–16 cells long, pointed at both ends, symmetrical, olive-green, walls thick but contents staining in phloxine mounts.

On: *Abies* sp.

TYPE and specimen examined: WASHINGTON: Mason Co.: Mt. Rose, Nov. 1949. Collected by C. J. Gould (WSC-PP *21430*).

Trimmatostroma pini W. B. Cooke, sp. nov.

Sporodochiis nigris, 0.5–1 mm. diam., superficialibus, sessilibus, centro depresso; hypostromate hemispherico, contextu irreguliter celluloso, hyalino vel brunnescente, ex hyphis parallele instructis, 2–3 μ diam.; sporis catenulatis, brunneis vel atro-brunneis, 0–3-septatis, lenibus, 9–12 × 18–30 μ.

Sporodochia black, 0.5–1 mm. in diameter, with a depressed center in older specimens, appearing like an immature *Tympanis*, sessile; sporodochium composed of hyaline to brown hyphae arranged in a dense body, interwoven below, parallel above, palisade-like, 2–3 μ in diameter; spores produced in simple chains, brown to brown-black, transversely septate, not connected by isthmi, the chains breaking up in mounting, opaque, spores mostly 4-celled but also commonly 1–3-celled, 9–12 × 18–30 μ, smooth-walled.

Host: *Pinus monticola* Dougl.

TYPE and specimen examined: WASHINGTON: Skamania Co.: Mt. St. Helens near Timberline Camp, Gifford Pinchot National Forest, 4500 ft., July 22, 1951. Collected by W. B. & V. G. Cooke (*28389*) and R. Binegar.

Kmetia corticola W. B. Cooke, sp. nov.

Sporodochiis pulvinatis, globosis, lutescentibus, 0.2–0.5 mm. diam., sessilibus, sparsis; sporophoris indistinctibus; sporis catenulatis, hyalinis, 3–5-septatis, 14–22 × 1.2–1.5 μ, septis indistinctis.

Sporodochia pulvinate, globose, lemon yellow when fresh to egg yellow when dry, appearing subgelatinous (when moist), 0.2–0.5

mm. in diameter, sessile, scattered; conidiophores, if present, breaking up into spore-like structures at maturity (the condition of the type); conidia produced in chains which break up, hyaline, embedded in a yellow, somewhat gelatinous material, 3–5-septate, 14–22 × 1.2–1.5 μ, septa indistinct, conidial chains appear to branch, isthmi between spores minute, indistinguishable in most cases.

On: Bark of a log of *Abies magnifica* Murr. var. *shastensis* Lem.

TYPE and specimen examined: CALIFORNIA: Lassen Volcanic National Park: Winter sports area between Checking Station and Sulphur Works, in Shasta fir forest, July 1, 1950. Collected by W. B. & V. G. Cooke (*26627*) and R. Binegar (WSC-PP *30130*).

7. STAMNARIA ON EQUISETUM

A number of collections of the imperfect stage of this fungus are filed in the Mycological Herbarium. Specimens from Washington and Idaho were collected on *Equisetum hyemale* L., *E. laveigatum* A. Br., and unidentified species of *Equisetum*. Of these, only one bears the perfect stage—a specimen collected at Pullman on June 29, 1895, by C. V. Piper (*384*) (WSC-PP *16229*). Morphologically, the spores of the *Titaeospora* stage in the Washington and Idaho material do not differ from such spores in collections made in Wisconsin, Germany, and elsewhere. However, the ascospores in the Piper collection measure 16–20 × 5–6 μ; it therefore agrees with *Stamnaria persoonii* (Moug. in Pers. ex. Fr.) Fckl. rather than with *S. americana* Massee & Morgan (spores 24–32 × 7–9 μ; Seaver, Inop. Disco. p. 230). *S. persoonii* is rarely reported in North American collections; indeed it is interesting to note that the most recent report was based on material also collected in the Pacific Northwest (Zeller, Mycologia **14**: 175. 1922). *S. americana* has been reported several times from the East and Midwest. Even though there is no difference between these two species in the *Titaeospora* stage, the differences in ascospore measurements are sufficient to maintain these species as distinct. The synonymy of the perfect and imperfect stages in the Northwest is presented below:

STAMNARIA PERSOONII (Moug. in Pers. ex Fr.) Fckl. Symb. Myc. 309. 1870.

Peziza persoonii Moug. in Pers. Myc. Eur. **1**: 288. 1822.

Peziza persoonii Moug. in Pers. ex Fr. Syst. Myc. **2**: 121. 1822.

Habrostictis persoonii (Moug. in Pers. ex Fr.) Rehm, Ascom. **13**: 615. 1881.

Stamnaria equiseti (Hoffm.) Sacc. Syll. Fung. **8**: 620. 1889.

TITAEOSPORA DETOSPORA (Sacc.) Bubak, Ann. Mycol. **14**: 345. 1916.

Lycoperdon equiseti Hoffm. Crypt. t. V. f. 1. 1790. (Pre-Friesian.)

Septoria detospora Sacc. Mich. **2**: 529. 1882.

Rhabdospora detospora (Sacc.) Allesch. in Sacc. Syll. Fung. **3**: 576. 1884.

Gloeosporium equiseti Ell. & Ev. Journ. Mycol. **1898**: 52. 1898.

Septogloeum equiseti (Ell. & Ev.) Diedicke, Krypt. Fl. Brandenb., Pilze **7**: 835. 1905.

It is possible that *Libertella equiseti* Desm. [Ann. Sci. Nat. Bot. Ser. 3, **8**: 179. 1847 = *Septoria equiseti* (Desm.) Desm. in Moug. & Nest. Stirp. Crypto. Vog. *1264*. 1850] is an older binomial for the imperfect stage. However, we have not had an opportunity to examine the type of *L. equiseti* Desm.

8. SOME FUNGI ASSOCIATED WITH GERMINATING SEEDS

In the summer of 1950, a number of interesting fungi were observed during germination trials conducted by Lloyd Hulbert[5] with seed of *Bromus* and *Tragopogon*. Mold development was more profuse on the seed of *Tragopogon* than on the seed of *Bromus*, but was serious on *Tragopogon* only if conditions were unfavorable for germination. Most *Bromus* seeds remained free of mold for a month or more unless kept uder very moist conditions. Dusting the seeds of *Tragopogon* with Spergon had little effect on the development of most of the molds, especially dematiaceous ones.

Specimens of these fungi are deposited in the Herbarium of the Department of Plant Pathology, The State College of Washington, Pullman.

TABLE I indicates the "seed host range" of some of the more interesting fungi found. Source of seed and conditions of growth will be discussed by Hulbert in another paper.

[5] We wish to express our appreciation to Mr. Lloyd Hulbert for his help with this section.

TABLE I

OCCURRENCE OF CERTAIN FUNGI ON GERMINATING SEEDS OF BROMUS AND TRAGOPOGON SPP.

Fungus \ Host	*Bromus*										*Tragopogon*				
	brizaeformis Fish. & Mey.	*catharticus* Vahl.	*commutatus* Schrad.	*mollis* L.	*patulus* Mert. & Koch	*racemosus* L.	*rigidus* Roth.	*squarrosus* L.	*sterilis* L.	*tectorum* L.	*dubius* Scop.	*micellus* Ownbey	*mirus* Ownbey	*porrifolius* L.	*pratensis* L.
Acremoniella verrucosa Togn.	+	−	+	−	−	−	+	−	+	+	−	−	−	−	−
Alternaria tenuis Nees (group)	+	+	+	+	+	+	+	−	+	+	+	−	+	+	−
Alternaria tenuissima (Fr.) Wiltshire	−	−	−	−	−	−	+	−	−	+	+	−	−	−	−
Aspergillus unguis (Emile-Weil & Gaud.) Th. & Rap.	−	−	−	−	−	−	+	+	−	+	−	−	−	−	−
Chaetomium convolutum Chivers	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−
Chaetomium funicolum Cooke	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−
Cladosporium herbarum (Pers.) Link	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
Delitschia bisporula (Crouan) Hansen	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
Helminthosporium cyclops Dreschl.	+	−	−	+	−	+	−	−	−	+	+	+	+	+	−
Hormodendrum cladosporioides (Fres.) Sacc.	+	−	−	+	−	−	−	−	−	+	+	−	+	+	−
Hormodendrum viride (Fres.) Sacc.	−	−	−	−	−	−	−	−	+	+	+	−	+	+	−
Memnoniella echinata (Riv.) Gall.	−	−	−	−	−	−	+	−	−	+	−	−	−	+	−
Penicillium expansum Link[6]	−	−	+	−	−	+	−	−	+	−	−	+	−	+	+
Stachybotrys atra Corda	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+
Stemphyllium consortiale (Thüm.) Groves & Skolko	+	+	−	−	+	−	−	−	−	+	+	−	+	+	−

[6] Identified by K. B. Raper.

Of the fungi listed in TABLE I, the following are worthy of additional comment.

The *Podosporiella* stage of *Pleosphaeria semeniperda* has previously been associated only with seed of various grasses. In germination tests conducted at the Seed Laboratory of the Agricultural Experiment Station at Pullman, the fungus has been found on *Poa pratensis* (WSC-PP *21048*). It has also been collected in Washington during extremely wet weather on caryopses of *Triticum aestivum* (WSC-PP *15924, 15925, 20371*). Dreschler, and Sprague (Manual of Diseases of Grasses, 1950) indicate that *Helminthosporium cyclops* is an occasional pathogen of various Gramineae. In pure culture this species usually develops spores on the agar surface as is normal with other species of *Helminthosporium*. However, with especially rich nutrients, elongate coremia may be produced on which spores similar to those of *H. cyclops* are produced at intervals on conidiophores. Dreschler and Sprague both considered this a nutrient variation. O'Gara found this form of the fungus on wheat seeds and placed it in *Podosporiella*. Later Brittlebank and Adam in Australia discovered the perfect stage at the base of the coremial bodies and named this *Pleosphaeria semeniperda*. While the perfect stage has been found only in Australian material, morphologically the *Helminthosporium* and *Podosporiella* stages are alike in both North American and Australian material. Consequently both these imperfect stages are assigned to *Pleosphaeria semeniperda*. The synonymy is given below:

Pleosphaeria semeniperda Brittlebank and Adam, Trans. Brit. Myc. Soc. **10**: 123–127. 1927.

Podosporiella verticillata O'Gara, Phytopath. **5**: 323–325. 1915.

Helminthosporium cyclops Dreschl. Jour. Agr. Res. **24**: 729. 1923.

The records of *Memnoniella echinata* are interesting since they place this important cellulolytic fungus north of the 45th parallel. According to a letter from W. L. White the previous northernmost record was from Ottawa, Ontario, Canada. The latitude of Pullman is approximately 46° 47′ or 1° 22′ north of Ottawa (latitude approximately 45° 25′).

A NEW SPECIES OF HETEROSPORIUM FROM SOIL [1]

R. G. ATKINSON [2]

(WITH 9 FIGURES)

An undescribed *Heterosporium* has been isolated from soil obtained from Ancaster, Ontario, Canada during the course of studies on the soil microflora in relation to potato scab (**3**). To the author's knowledge, the only other account of the isolation of a member of this genus from soil is that of Chand (**1**) in India who reported *H. allii* Ell. & Mart. from this habitat.

Jacques (**2**) has published the first monograph of the genus and in it he describes seven common species. Dr. Jacques kindly examined a culture of the present fungus and in correspondence stated, "It approaches *Heterosporium cladosporioides* Ell. & Ev. (Proc. Acad. Nat. Sc. Phil. **1894**. 382) but the hyphae and conidiophores are much narrower, the conidia somewhat smaller and decidedly constricted in your material. The conidia are said not to be constricted in *Heterosporium cladosporioides* and in fact they are slightly broader toward the middle." He concluded that the present fungus represents a new species of *Heterosporium*.

Heterosporium terrestre sp. n.

Coloniae tholiformes, brunneo-nigrae, velutinae, olivaceo-brunnescentes, leniter crescentes; hyphae septatae, cylindricae, pallide luteo-olivaceae, sed irregulariter inflatae et in stromate pallide brunneo-olivaceae, 1.5–4 μ dia., angulis rectis vel fere rectis ramosae; stroma nigrum, superficiale, convexum, 400 μ lat. vel minus, pseudoparenchymaticum; conidiophora ab hyalinis pallide luteo-olivacea, si lateralia a brevissimis cylindricis vel doliformibus vel subclavatis longiora gracilia, a cylindricis clavata, a rectis subarcuantia, in apice interdum capitata vel angulo recto deflexa, si terminalia saepius clavata, 2–31.5 × 1.5-2.5 μ, parte principe continua vel caudata, a septo in-

[1] Contribution No. 1156 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Associate Plant Pathologist.

ceptis, saepius et nonnunquam conspicue geniculatis, simplicibus vel interdum ramosis angulis aliquoties rectis; conidia 3.5–10.5 × 2.5–5 μ, med. 6.5 × 3 μ, trimorpha, parva, pallide luteo-olivacea, singula in sterigmatoidea cauda, ab acrogenis pleurogenescentia, ad 10 in conidiophoro singulo, ab echinulatis aculeata, basi attenuata in pedicello brevi; typi conidiorum sunt: (1) ab ovalis ovata, continua vel uniseptata, 3.5–7 × 2.5–5 μ, med. 6 × 3 μ, (2) breviter cylindrica, continua vel uniseptata, 5–7 × 2.5–3.5 μ, med. 6 × 2.5 μ, (3) a soleaeformibus elongato-cylindrica, saepius uniseptata, interdum bi- vel triseptata, typice nec tamen semper in septo constricta, 7–10.5 × 2.5–3.5 μ, med. 8 × 3 μ; typi (2) et (3) interdum cellula basali latiore.

Colonies on potato dextrose agar dome-shaped, brownish-black, velvety, gradually covered with fine, pale, "olive buff" aerial mycelium and later between "light brownish olive" [3] and "brownish olive," at first smooth, becoming slightly rough with age, slow-growing; hyphae septate, cylindrical, pale yellowish olive but irregularly swollen and pale brownish olive in the stroma, brownish in mass, 1.5–4 μ diam., branching at right angles or nearly so, frequently wavy or in spirals when growing submerged in agar; stroma black, superficial, convex, up to 400 μ wide, pseudoparenchymatous; conidiophores hyaline to pale yellowish olive, lateral, very short, cylindrical, doliform, or slightly clavate, to longer and slender, cylindrical to clavate, straight or slightly curved, the end occasionally knob-like or bent at right angles, or terminal and then usually clavate, on aerial mycelium or stroma, length extremely variable, 2–31.5 × 1.5–2.5 μ, av. 9 × 2 μ, composed of a continuous primary portion only, or with prolongations usually separated by septa and often at various angles resulting in a pronounced zigzag effect, usually geniculate, scars clearly visible only under high magnification, simple or occasionally branched, sometimes at right angles; conidia smaller than in other species of *Heterosporium*, pale yellowish olive, borne singly, usually in groups of two or more, on sterigmatoid projections which occasionally remain attached to the spore as a long tail, acrogenous, becoming pleurogenous as the conidiophore elongates, as many as 10 conidia observed on one conidiophore, echinulate to aculeate, may be partly verrucose in old cultures, base tapering to a short, not always clearly visible, pedicel, of three types; (1) oval to ovate, continuous or 1-septate, 3.5–7 × 2.5–5 μ, av. 6 × 3 μ, (2) short-cylindrical, continuous or 1-septate, 5–7 × 2.5–3.5 μ, av. 6 × 2.5 μ, (3) long cylindrical to sole-shaped, usually 1-septate, occasionally 2- or 3-septate, typically constricted at the septum but not always, 7–10.5 × 2.5–3.5 μ, av. 8 × 3 μ, in types (2) and (3) the end

[3] Ridgway, R. Color Standards and Color Nomenclature, Washington, D. C. 1912.

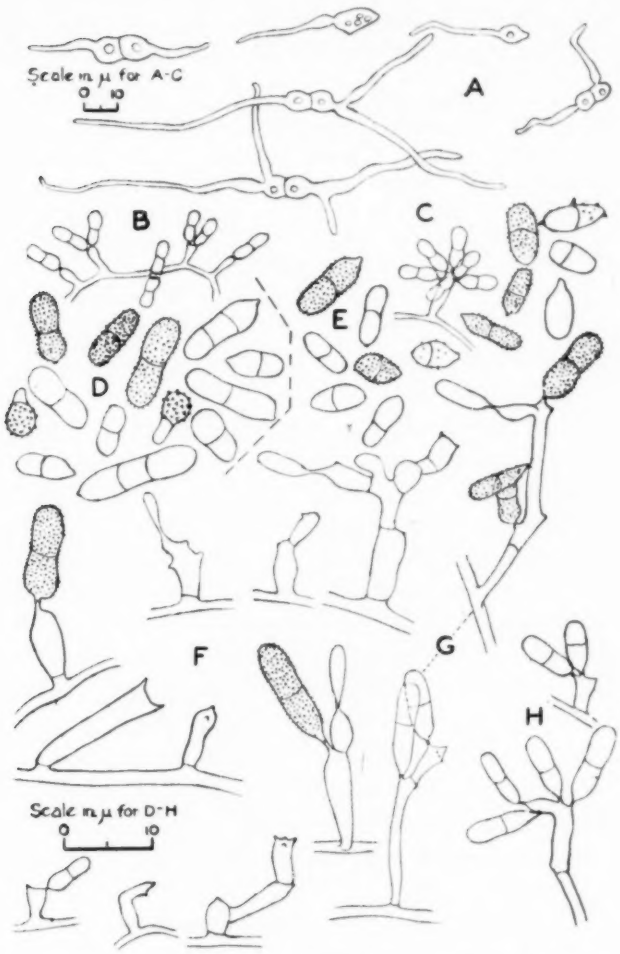


FIG. 1. Drawn with the aid of a camera lucida, A–C × 500, D–H × 1300. A. Method of spore germination. B–C. Conidiophores, with conidia, in soil-extract-agar culture. D. Conidia from a soil-extract-agar culture. E. Conidia from a potato-dextrose-agar culture. F. Conidiophores, some with conidia, in a potato-dextrose-agar slide culture. G. Conidiophores, some with conidia, in a potato-dextrose-agar slide culture. H. A lateral and a terminal conidiophore, with conidia, in a potato-dextrose-agar slide culture.

rounded, sometimes the basal cell broader, range of three types, 3.5–10.5 × 2.5–5 μ, av. 6.5 × 3 μ.

Isolated from soil obtained from Ancaster, Ontario, Canada on October 31, 1947.

Type: a dried potato dextrose agar plate culture has been deposited in the Mycological Herbarium, Division of Botany and Plant Pathology, Ottawa, Canada, DAOM *28282*.

Subcultures have been deposited with the American Type Culture Collection, Washington, D. C., the Centraalbureau voor Schimmelcultures, Baarn, Holland, and the Commonwealth Mycological Institute, Kew, Surrey, England.

MATERIALS AND METHODS

The fungus was isolated from soil dilutions plated on acidified soil extract agar.[4] The isolate was maintained, with occasional transfers, on soil-extract-agar slants in an ordinary refrigerator from 1947 until the present studies were initiated in 1951. At this time the fungus was purified by monosporous isolations, as transfer to potato dextrose agar from the soil extract agar stock culture showed the latter to be contaminated. Six monosporous isolates, each growing on potato-dextrose-agar and on soil-extract-agar slants, served as the source material for the present study.

Measurements, drawings, and photomicrography of the small, inconspicuous conidiophores were greatly faciliated by growing the fungus on slide cultures of potatoes dextrose agar, and of soil extract agar. Spore suspensions of the organism were spread over thin layers of the medium on sterile microscope slides which were attached to small, wooden labels resting, under aseptic conditions, on moist filter paper in Petri dishes. These were then incubated at 25°–27° C.

Spore germination was studied in non-sterile redistilled water by means of van Tieghem cells.

Purified isolates were obtained by picking up single germinat-

[4] 1000 gms. dry soil and 1 liter water autoclaved 30 mins. at 15 lbs. pressure, filtered under suction while hot through No. 3 Watman filter paper, 15 gms. agar added to filtrate and water up to 1 liter, 0.2 gm. K_2HPO_4 added as buffer, sterilized at 15 lbs. pressure, 20 mins., acidified with lactic acid just before using.

ing spores from plates of potato dextrose agar on which loopfuls of a spore suspension had been allowed to run down parallel paths. A variant, which arose spontaneously in culture and which sporulated very sparsely, was isolated by using a suspension of mycelium in the above procedure and picking up small, germinating, hyphal fragments.

SPORE GERMINATION

Spore germination was studied at 3°, 15°, 24°, 30° and at 33°–35° C. After 16 hours, germination was mostly terminal and appeared to be optimum at 24° C, at which temperature most of the spores showed one germ tube, and a few two, one from each end. Many of the germ tubes after 16 hours had branched once, nearly at right angles, and frequently at, or close to, the point of emergence of the tube from the spore. A few showed two branches, one from each germ tube.

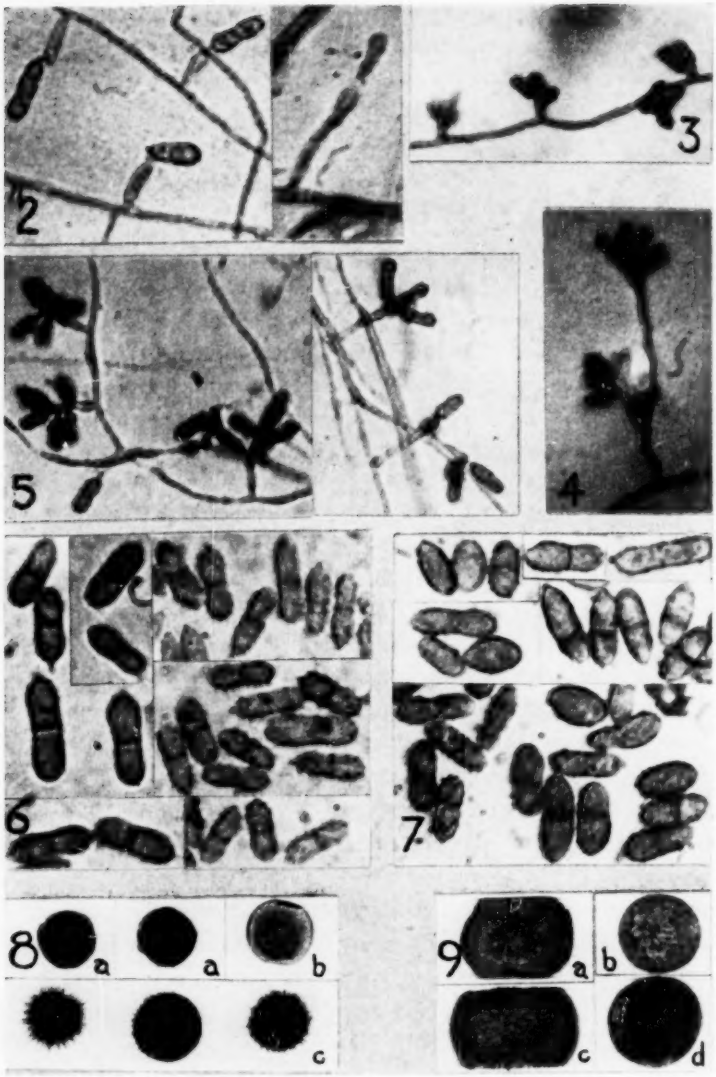
Germination at 30° C was similar to that at 24° C but the germ tubes did not branch. At 33°–35° C the tubes were very short, most of them being not more than two or three times the length of the spore. The majority of spores showed a single germ tube but many had produced two, one from each end of the spore. At 15° C germination was similar to that at 33°–35° C. The process was very slow at 3° C and after 65 hours only a small, bulbous protuberance appeared at either one or both ends of the spore. After 7 days the longest germ tube was not more than two or three times the length of the spore.

In a second test the percentage germination was determined. Only 43% of the spores germinated at 3° C, whereas 92%–98% of them germinated, with no consistent variation, at 19°, 22°, 25°, 30°, and 33–35° C. Clearly, most spores produced by this fungus will germinate over a wide temperature range.

CULTURAL CHARACTERISTICS

On Potato Dextrose Agar

Radial growth was slow on this medium, the diameter of colonies grown from single spores at 27°–29° C reaching only 3



FIGS. 2–7. Photomicrographs. 2–5, × 645; 6, 7, × 1455; 8, 9, × 5/6; P.D.A. = potato dextrose agar, S.E.A. = soil extract agar. 2–5. Conidiophores with conidia; 2, on P.D.A. slide culture; 3, 4, simple and branched, respectively, on aerial hyphae on S.E.A. plate culture; 5, on surface of

mm. after 5 days and 1.5 cm. after 15 days. Colonies derived from spore inoculum consisted, after 4 days, of very pale, "olive buff" aerial mycelium. Gradually the growth darkened and became dome-shaped as seen in FIG. 8-a. In contrast, colonies grown from mass mycelial inoculum were brownish black from the start. The dark, dome-shaped appearance of the colonies was due to the compacting of hyphae into sharply pointed mycelial strands of approximately equal length which grew, densely packed, from, and at right angles to, the convex stroma. This resulted in a relatively smooth, velvety, hemispherical profile. Regardless of the kind of inoculum, typical colonies of the fungus eventually became paler as "olive buff" aerial mycelium grew between and over the dark strands.

On Soil Extract Agar

Growth on this carbohydrate-deficient medium never became dense or dome-shaped. A stroma was absent. Only a few thin mycelial strands projected upward and over these grew a pale "olive buff" cottony mycelium. This central aerial portion was surrounded by a much larger area of appressed, "light brownish olive" growth.

An interesting effect of this medium on the size and morphology of the spores was noted. Only two types were formed, (1) short cylindrical and (2) long cylindrical to sole-shaped, in contrast to the three types formed on potato dextrose agar, on which oval to ovate spores were also produced. Furthermore, the spores from soil-extract-agar cultures tended to be slightly longer and narrower than those from potato-dextrose-agar cultures. These differences are shown in TABLE I.

Conidiophores produced on soil extract agar were similar to those formed on potatoes dextrose agar.

S.E.A. plate culture. 6, 7. Conidia from S.E.A. and P.D.A. cultures, respectively. 8-a, b. 11-day-old monosporous colonies on P.D.A.: a, wild type; b, 'grey variant'; c, 12-day-old colonies of 'bristly variant' on P.D.A. grown from single hyphal fragments. 9. P.D.A. slant cultures: a, wild type, 15 days old; b, 'grey variant,' 11 days old; c, d, 'bristly variant,' 15 days old and 11 days old, respectively.

VARIATION IN CULTURE

The organism showed a ready capacity to change in culture and two stable variants have been isolated in the present study. The first variant appeared as a blackish, bristle-like growth in the first subcultures of each of the monosporous isolates that were used as the source material for the present study and had been growing on potato-dextrose-agar slants for approximately two months. When, after about one month, the subcultures were transferred to fresh slants the bristle-like growth still persisted. Because of its appearance this variant will be referred as the 'bristly variant.'

TABLE I

COMPARISON OF THE MORPHOLOGY AND SIZE OF SPORES IN *Heterosporium terrestre* GROWN ON POTATO DEXTROSE AGAR AND ON SOIL EXTRACT AGAR

Spore morphology	Potato dextrose agar		Soil extract agar	
	Range	Av.	Range	Av.
Oval to ovate	3.5–7×2.5–5 μ	6×3 μ	not formed	—
Short cylindrical	5–7×2.5–3.5 μ	6×2.5 μ	5–7×2–3 μ	7×2.5 μ
Long cylindrical to sole-shaped	7–10.5×2.5–3.5 μ	8×3 μ	8–14×2–3.5 μ	9.5×3 μ
All types	3.5–10.5×2.5–3.5 μ	6.5×3 μ	5–14×2–3.5 μ	8.5×3 μ

In order to separate the wild type from the 'bristly variant,' single spore isolations were made from one of the second series of subcultures. Two of these monosporous isolations gave rise to a second variant subsequently referred to as the 'grey variant,' the remaining thirteen isolates developing into typical wild type colonies. The two types of variants which arose spontaneously in wild type cultures of the fungus are described below.

The 'Bristly Variant'

The 'bristly variant' was obtained in a pure state by isolating very small, germinating hyphal fragments. After three days, these developed into small colonies of fine, pale, "olive buff" aerial mycelium as in the wild type. Like the latter, the variant became darker, but produced a different kind of growth. At 12 days the

colonies were dome-shaped, blackish, and composed of long, slender, quill-like mycelial strands. In contrast to the smooth, velvety appearance of the wild type, the profile of the variant colony resembled the quill-covered back of a porcupine, as seen in FIG 8-c. Gradually, fine, pale, aerial mycelium developed between the quill-like structures. In slant cultures, the bristle-like growth in the outer portion of the colony eventually became effused and "deep grayish olive" in color. The 'bristly variant' produced only a very few spores and did not form a stroma.

On soil-extract-agar slants this variant produced a sparse, pale, "olive buff," aerial mycelium surrounded by a wide, appressed, "light brownish olive" outer portion. As on potato dextrose agar, only a few spores were formed.

The 'Grey Variant'

This variant was first recognized when two monosporous isolates, whose origin has been previously mentioned, developed into "grayish olive," dome-shaped colonies of felt-like appearance, as seen in FIG. 8-b. When transferred to potato-dextrose-agar slants this variant, at 4 days, produced a "dark grayish olive" colony composed of long, blackish, slender mycelial strands which were flexuous and widely separated. Soon fine, pale, "olive buff" aerial mycelium developed over the strands and the colonies became "deep grayish olive" and felt-like in appearance. This variant, like the 'bristly variant,' did not produce a stroma, but in comparison sporulated moderately well, although not nearly as abundantly as the wild type.

On soil extract agar the 'grey variant' produced a sparse, cottony, "olive buff" aerial mycelium surrounded by an almost colorless to "light brownish olive," appressed outer portion. Spore production, as on potato dextrose agar, was moderate in relation to that of the wild type.

ACKNOWLEDGMENTS

The author wishes to thank Dr. J. Émile Jacques of the Montreal Botanical Garden, Montreal, Quebec who kindly examined a cul-

ture of the present *Heterosporium* and recognized it as a new species. It is a pleasure to acknowledge the assistance of Dr. B. Boivin, Botanist, Taxonomy Section, Division of Botany and Plant Pathology, Dept. of Agriculture, Ottawa, Canada, who translated the species diagnosis into Latin.

LABORATORY OF PLANT PATHOLOGY,
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A NEW SPECIES OF FOMES FROM PAKISTAN

S. Murtaza Hussain [1]

(with 2 figures)

In a previous paper, the author (**1951**) indicated the presence of a new form of *Fomes conchatus* (Pers. ex Fr.) Gill., but on referring the specimens to Kew, it was decided to describe it as a new species. It is now proposed to name this *Fomes ajazi*, and its description is given below. It differs from *Fomes conchatus* (Pers. ex Fr.) Gill., in having radiately rugose and strigate upper surface and somewhat smaller spores (Figs. 1, 2f).

Fomes ajazi sp. nov.

Sporophora sessilis, dimidiata, in juventute suberosa, dein leviter lignosa, 5.5–9 × 3–7 cm., 1–3 cm. crassa, marginem attenuans. Contextu ferrugineo vel ferrugineo-cinnamoneo, suberoso, 0.25–1.0 cm. crasso. Hyphis dilute fulvis, 1.7–4.6 μ, septatis, paucis ramis, sine fibulis, crasse tunicatis, 0.2–0.4 μ crassis, interdum usque ad 1.16 μ; hyphis hyalinis tenuiter tunicatis usque ad 4 μ. Superficie summa aspera, fulvo-ferruginea vel fulvo-brunnea cum colore griseo, distincte concentrice zonata, radialiter rugosa et strigosa cum pilis appressis radialiterque collocatis et margine 1.5 cm. lato, interdum latiore, distincte ferrugineo vel dilute ferrugineo. Hyphis superficiei summae 3–4.6 μ ferrugineis, crebro tunicatis, parce septatis et paucis ramis, sine fibulis. Superficies hymenialis ferruginea colore leviter diluto, glabra velutinaque cum fulgore nitido. Poris angulatis, plerumque sexangulis, quinquangulis vel interdum rotundatis, minutis, 69–150 × 58–127 μ. Tubulis pororum fulveolis vel dilute fulveolis, stratosis, 0.5–2.0 mm. Hyphis pororum crasse tunicatis, fulvis, septatis, ramis paucis, 1.7–2.5 μ, sine fibulis, 0.2–0.3 μ crassis. Setis numerosis, fulvis, crasse tunicatis basi inflatis, 16–23 × 6–10 μ. Margine dilute fulveolo. Hyphis marginis 1.7–2.5 μ, dilute fulveolis, parce septatis, paucis ramis, sine fibulis, 0.2–0.3 μ crassis. Basidiis hyalinis, forma clavarum, 7–9 × 5–6 μ. Sporis dilute fulveolis, ellipsoideis glabris 3.4–4.3 × 2.5–3.4 μ.

Invenitur ad altitudinem pectoris vel demissiorem, in *Lonicera quinque-*

[1] Research Assistant, Pakistan Forest Research Institute, Abbottabad, N.W.F.P.

loculari Hardw., in silvis Patriatis et vicinitate Upper Topa, Murree Hills, ad 6000–7000 pedum altitudine.

Sporophore sessile, shelving, semi-circular, corky when young, slightly woody later, 5.5–9 × 3–7 cm., 1–3 cm. thick, thinning out towards the margin. Context brown or cinnamon-brown, corky, 0.25–1.0 cm. Context hyphae pale brown, 1.7–4.6 μ, septate, sparingly branched, without clamps, thick-walled, wall 0.2–0.4 μ thick, sometimes up to 1.16 μ, hyaline hyphae thin-walled, up to 4 μ. Upper surface rough, dark brown or snuff brown with greyish

FIG. 1. Photograph showing upper and lower surface of the sporophores of *Fomes ajazi* Hussain sp. nov. (Size of specimens 5.5 × 3.5 cm.).

tinge, distinctly concentrically zoned, radiately rugose and strigate with adpressed radially aligned brown hairs, and a distinct brown or light brown border, 1.5 cm. or more wide. Hyphae of the upper surface 3–4.6 μ, brown, thick-walled, sparingly septate and branched, without clamps. Hymenial surface brown with slightly pale tinge, smooth, velvety, with a silky sheen. Pores angular, usually hexagonal, pentagonal or sometimes circular, minute, 69–150 × 58–127 μ. Pore tubes brown or pale brown, stratified, 0.5–2.0 mm. Hyphae in the pore tubes thick-walled, brown, septate, branching scanty, 1.7–2.5 μ, without clamps, wall 0.2–0.3 μ thick. Setae abundant, brown, thick-walled, with bulbous base, 16–23 × 6–10 μ.

Margin pale brown. Hyphae of the margin 1.7–2.5 μ, pale brown, sparingly septate and branched, without clamps, wall 0.2–0.3 μ thick. Basidia hyaline, club-shaped, 7–9 × 5–6 μ. Spores pale brown, ovoid, smooth, 3.4–4.3 × 2.5–3.4 μ.

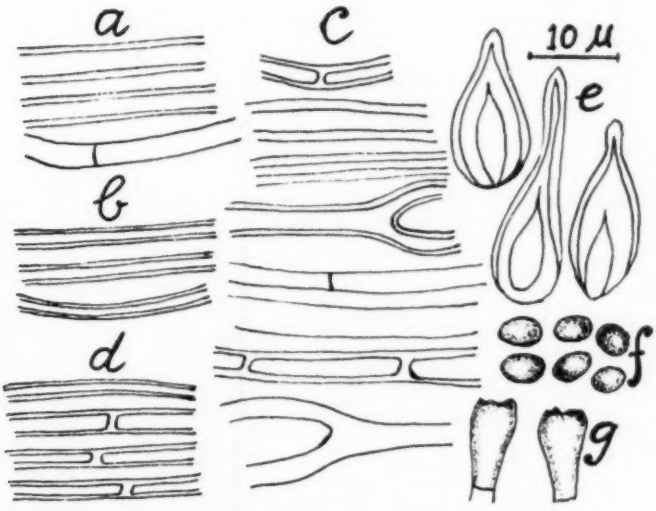

FIG. 2. a–d, Camera lucida drawings showing the hyphae from the upper surface margin, context and hymenial surface of the sporophore of *Fomes ajazi* Hussain sp. nov., respectively. Thick- and thin-walled hyphae are clear. e, f, g. Setae, spores and basidia respectively.
All drawings made with camera lucida. Magnification × 860.

Found at breast height or lower on *Lonicera quinquelocularis* Hardw., in the Patriata forests and in the forests in the vicinity of Upper Topa, Murree Hills, 33°–34° N., 73°–74° E., between 6000–7000 feet, or higher.

The fungus is named in honor of my late father, S. Ajaz Hussain. Specimens have been deposited in herbaria of the Royal Botanic Gardens, Kew, and the Pakistan For. Res. Inst.

ACKNOWLEDGMENT

The author is deeply indebted to Mr. S. A. A. Anvery, P.Ft.S., Director, Pakistan Forest College and Research Institute, for encouragement, and heartfelt thanks are due to Dr. N. L. Bor and

Miss E. M. Wakefield of Kew, for very kindly checking the specimens and confirming the identity, and to Father J. Klaver, for very kindly translating the description into Latin. Hearty thanks are also due to my sister for help in arranging the data and to Messrs. Irfan, Kazmi and Athar, for cooperation in collecting the specimens.

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REFERENCE

Hussain, S. Murtaza. 1951. Some observations on *Fomes conchatus* (Pers. ex Fr.) Gill. Pakistan Jour. Forestry 1: 149.

SPECIES OF SYNCHYTRIUM IN LOUISIANA. VII. A NEW SPECIES ON URTICA CHAMAEDRYOIDES

MELVILLE T. COOK

(WITH 5 FIGURES)

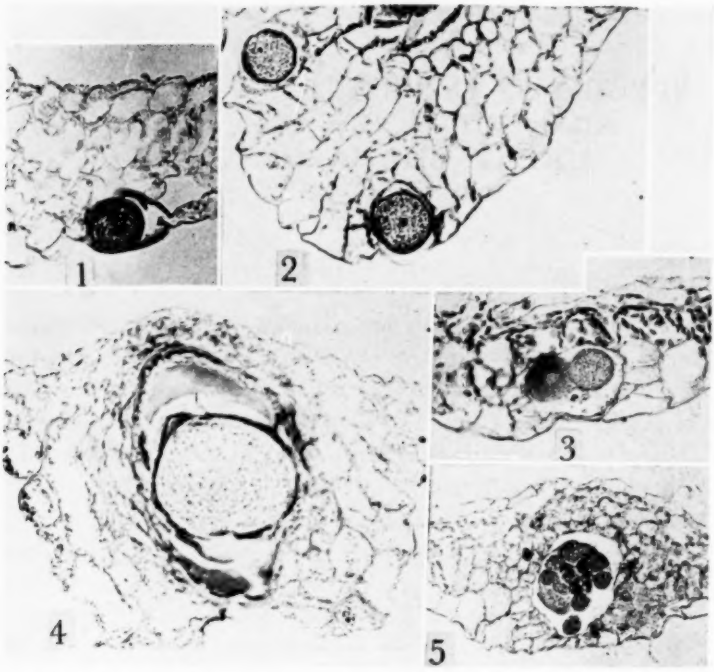
The material for this study was collected a few miles from Baton Rouge in March 1950. The plants were in full bloom and the fungus was found in abundance on leaves, stems, and flowers. The deformities of the infected parts make them rather conspicuous. The material was found in a forest area that had been flooded for a short time a few days earlier and the growth of the host plants had been very rapid. The mature galls were numerous, usually hemispherical and the base of each was partially submerged in the tissues of the host plant.

The infections start in the epidermal cells (FIGS. 1, 2, 3) of young organs, especially on the under surfaces of the leaves. The infected cells enlarge rapidly; much more rapidly than the fungus. The galls are formed by growths of the cells from the epidermal cells. The cells of the galls are unusually large (FIGS. 2, 3). The epidermal cells of the galls frequently become infected and conspicuous, compound galls are formed (FIG. 2). The opening over the infected cell was usually large (FIGS. 1, 2, 3, 4). The fungus may die but the organs are more or less deformed. The content of the host cell is not abundant. Multiple infections are common (FIG. 3). The host cell nucleus (FIG. 3) disappears early.

The fungus is granular and rarely fills the host cell completely. The cell wall around the fungus is variable in thickness but disappears before the sporangia are completely formed. The sporangia (FIG. 5) are about 40 in number and variable in size.

Synchytrium urticae sp. nov.

Gallis copiosis in stirpibus foliis floribusque; sphaericis; 48 × 48 usque ad 93 × 156 μ; soris subflavis in colorem fulvum se mutantibus; 39 × 39 μ; spo-



FIGS. 1–5. *Synchytrium urticae*. 1. Early infection in epidermal cell of the host plant. 2. Same, but the gall is enlarged and compounded by later infections. 3. Multiple infection and host cell nucleus. 4. Fungus and gall very much enlarged. 5. Gall showing sporangial stage of fungus.

rangiis numero circa quadraginta et $8 \times 15\,\mu$ diametro. Hab. *Urtica chamaedryoides*, Baton Rouge, Louisiana, U. S. A.

Galls abundant on stems, leaves and flowers; more or less spherical; 48×48 to $93 \times 156\,\mu$. Sorus light yellow becoming dark yellow; $39 \times 39\,\mu$. Sporangia about 40 in number and $8–15\,\mu$ in diameter.

The writer wishes to express his thanks to Dr. S. J. P. Chilton for encouragement in making these studies, to Dr. C. W. Edgerton and Dr. E. C. Tims for making the photographs and to Dr. P. J. Moorehead for preparing the Latin diagnosis.

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NOTES AND BRIEF ARTICLES

The ability of Coprini to sensitize man to ethyl alcohol

George P. Child a publié dans *Mycologia* (**1952**, p. 200) un article dont la conclusion est déjà résumée par le titre: "The inability of Coprini to sensitize man to ethyl alcohol."

Coprinus atramentarius, C. comatus, and *C. micaceus* furent consommés et à la suite de leur ingestion, une boisson alcoolique fut absorbée. Aucun signe pathologique ne fut observé. Par contre, une certaine rubéfaction faciale fut notée après l'absorption de *Panaeolus campanulatus,* non accompagnée d'alcool d'ailleurs. L'Auteur conclut que les cas d'intoxication attribués à la consommation conjuguée de *C. atramentarius* et d'alcool avaient peut-être été provoqués par l'ingestion de *Panaeolus* pris pour des Coprins.

Je puis assurer: 1°) qu'en France, tout au moins, *C. atramentarius* produit fréquemment de la rubéfaction faciale (s'étendant parfois sur tout le sommet du buste), accompagnée, dans les cas extrêmes, d'une intolérable accélération du rythme cardiaque. La détermination de l'espèce coupable a été vérifiée dans la plupart des cas par un mycologue compétent et la confusion avec un Panaeole est absolument exclue.

2°) Que l'absorption de vin (ou de boisson alcoolique) sans être toujours nécessaire à l'apparition du syndrome, le renforce énormément.

3°) Que l'absorption de vin au cours des repas suivant celui où le Coprin a été consommé peut parfois déclencher la réapparition des symptômes, sans que le Coprin soit consommé à nouveau. Cette réapparition du syndrome par simple prise d'alcool peut se produire plusieurs fois de suite, ceci jusque pendant 2 ou 3 jours.

Les affirmations ci-dessus sont basées sur une littérature mycotoxicologique abondante que George F. Child semble ignorer et qui contient des indications précises. Je citerai seulement:

J. Chifflot, Sur un cas de rubéfaction de la face, tendant à se généraliser, à la suite de l'ingestion de *Coprinus atramentarius* Fr. (Bull. Soc. Myc. de France, **1916**, p. 63.)

et surtout :

A. POUCHET, Troubles circulatoires causés par l'absorption consécutive de Coprins et de vin. (Bull. Soc. linnéenne de Lyon, **1927**, p. 59.)

mais il existe plusieurs autres études sur cette question.

J'ajoute que j'ai dans mes notes inédites des observations d'intoxication par *C. atramentarius* + alcool (le champignon a été identifié par moi-même). Je ne les jamais publiées car elles m'ont paru sans aucun intérêt, étant donné le grand nombre de cas de ce genre cités dans les Revues mycologiques françaises. On ne saurait mettre ce singulier phénomène en doute ; c'est dire que George P. Child a eu grandement raison de faire preuve de prudence dans ses conclusions et d'écrire : "It is possible, however, that no mistake had been made and that Coprini collected from other sites or fed to other subjects might have alcohol-sensitizing effects."

Il me reste à indiquer qu'*aucune* des hypothèses avancées pour expliquer ce type d'intoxication ne satisfait l'esprit : anaphylaxie ? Non, car il y a eu des cas d'intoxication chez des personnes consommant cette espèce pour la première fois. Idiosyncrasie (sensibilité individuelle) ? Non, car j'ai observé des cas d'intoxication collective. Mutation toxique chez certains mycéliums de *C. atramentarius* ? Non, car ma femme a accepté de consommer (en l'accompagnant de vin) le reste d'une récolte qui avait rendu malade deux personnes. Or, elle n'éprouva aucun symptôme fâcheux.

Quant au rapprochement de l'intoxication atramentarienne et de l'action de l'Antabus, il vient immédiatement à l'esprit, du fait d'une certaine communauté de syndrome ; il a été envisagé, notamment, par un de mes amis qui a beaucoup étudié cette nouvelle thérapeutique anti-alcoolique, mais je ne crois pas que cette hypothèse soit entrée dans le domaine de la vérification expérimentale ou chimique.

En résumé : La toxicité, non constante mais fréquente, de *Coprinus atramentarius*, surtout et presque exclusivement avec adjonction d'alcool, est incontestable, au moins en Europe occidentale. De nombreux cas en ont été signalés dans les publications françaises.

Il ne semble pas qu'une explication vraiment satisfaisante de cette curieuse forme d'intoxication ait encore été donnée.

Un rapprochement avec l'action de l'Antabus n'est peut-être pas à exclure *a priori*, mais rien ne permet encore de l'affirmer.—MARCEL JOSSERAND (Lyon, France, Mai, 1952).

CORRECTION

In the paper by Alexopoulos and Cation: *Gnomonia fragariae* in Michigan, p. 221 (March-April), the name *Gnomonia fragariae* as used in footnote 3 should be replaced by *Gnomonia fructicola* (Arn. & Arn.) Fall.

DIE PILZE MITTELEUROPAS

Mycologists interested in the larger fungi will be glad to know that this monumental work, interrupted by the war, is to be continued. The publication is now in the hands of the firm of Julius Klinkhardt, Bad Heilbrunn, Oberbayern, which announces Volume III, Die Russulae, by Julius Schaeffer, in a somewhat revised form from the scarce first printing but with all the original colored plates.

LABORATORY TRAINING COURSES

The Communicable Disease Center of the U. S. Public Health Service Chamblee, Georgia, announces a series of laboratory training courses for the year 1953, of which the following are of special interest to mycologists:

Jan. 5–9. Laboratory Diagnostic Methods in Veterinary Mycology.
Mar. 16–27. Laboratory Methods in Medical Mycology. Part I. Cutaneous Fungi.
Mar. 30–Apr. 10. Laboratory Methods in Medical Mycology. Part II. Subcutaneous and Systemic Fungi.
Nov. 23–27. Laboratory Methods in Medical Mycology.

Further information and application forms may be secured by addressing Laboratory Training Services, Communicable Disease

Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

RESEARCH GRANTS OF THE MYCOLOGICAL SOCIETY OF AMERICA

The Mycological Society of America has decided, through its Council, to make available to its members the annual income received from its sustaining members "to promote interest in the field of mycology, to further research and to serve as a special publication fund." The honorarium of the speaker for the Annual Lecture is to be paid from this fund, and the remainder is to be used for grants-in-aid. It is suggested that grants be limited to $100. Application blanks may be obtained from Dr. Leland Shanor, Secretary-Treasurer (Department of Botany, University of Illinois, Urbana, Illinois). Applications, submitted in triplicate to the Chairman of the Research Grants Committee, should be in the hands of the Chairman by February 1, 1953.

Members of the Committee: John N. Couch, University of North Carolina; Ralph Emerson, University of California; Grant D. Darker, Chairman, Committee on Research Grants, Ben Venue Laboratories, Inc., Bedford, Ohio.

MYCOLOGIA INDEX

The index to volumes 1–24 of MYCOLOGIA, of which the supply has for some time been believed exhausted, is again in stock, and can be furnished, in paper covers, at $2.50 by The New York Botanical Garden.

During the year 1952, the Mycological Society of America has lost five members by death. A brief notice of Miss Burlingham's death appeared in the May-June issue.

Dr. W. R. Ivimey Cook died in Britain on January 2 at the age

of 51. Dr. Cook was best known to American mycologists for his work on the Plasmodiophorales and other soil and root fungi.

Dr. W. Lawrence White died at Concord, Massachusetts, on July 31, as the result of an automobile accident, at the age of 44. As director of the Farlow Herbarium, as an active member of the Society and of the Microbiological Section of the Botanical Society, he was well known to a large number of mycologists in the United States.

Dr. Samuel C. Damon II died at Kingston, Rhode Island, on August 8, at the age of 28. Dr. Damon had just begun what promised to be a fruitful investigation on certain imperfect fungi.

Dr. Melville T. Cook died in Washington, D. C., on August 11, at the age of 83. In his earlier years he was particularly active in the study of tropical and temperate plant diseases. Later, he became interested in viruses and then in the genus *Synchytrium* and continued his study of the latter almost until his death.

It is hoped that it will be possible to print suitable biographical notices of all these in the near future.

REPORT OF THE MANAGING EDITOR OF MYCOLOGIA

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED JUNE 30, 1952

Cash receipts

Joint funds

Members' subscriptions (Mycological Society)	$2,536.00	
Other subscriptions	4,335.98	
Sales of v. 25 and later	723.47	
Excess pages and illustrations	104.97	
	7,700.42	

Special funds

Sales of V. 1–24 and Index	93.95	
Income from endowment	725.00	
	$ 818.95	
Total receipts		$ 8,519.37

Cash disbursements

Printing and distribution (*Note*)	$9,289.59	
Office expense	250.28	
Total expenditures		$ 9,539.87
Excess of expenditures over receipts		1,020.50
Unexpended balance July 1, 1951		7,022.44
Unexpended balance July 1, 1952		$ 6,001.94
Endowment		$14,000.00

Note: Printing and distribution includes $1,318.21 expense of the May-June, 1951, issue, which was paid in July 1951.

The above MYCOLOGIA funds are administered by The New York Botanical Garden. The balance at July 1, 1952, is in agreement with the amounts shown in the financial statements of The New York Botanical Garden, which have been examined and reported on by Price Waterhouse & Co. as of June 30, 1952.

To summarize very briefly: On July 1, 1951, MYCOLOGIA had an excess of receipts over expenditures of $2,372.96, not yet having paid for the May-June number. If that expense of $1,318.21 had been paid for by July 1, 1951, the excess of receipts over expenditures would have been $1,054.75 (after publishing 6 issues totaling 753 pages). On July 1, 1952, MYCOLOGIA had an excess of expenditures over receipts of $1,020.50, having paid for the extra May-June number. Deducting that $1,318.21 from the year's expenditures would leave an excess of receipts over expenditures for 1951–52 of $297.71 (after publishing 6 issues totaling 791 pages).

DONALD P. ROGERS, *Managing Editor*

REVIEWS

STUDIES IN THE SCLEROTINIACEAE. I. TAXONOMY OF THE SCLEROTINIACEAE, by N. Fabritius Buchwald. Kgl. Veterinaer- og Landbohøjskoles Aarsskrift **1949**: 75–191. 7 fig. (Contr. Dept. Pl. Path. no. 32). Copenhagen, 1949.

This paper, together with the author's earlier article published in Friesia in 1947, constitutes an outstanding contribution to the taxonomy of the Discomycetes. American mycologists are fortunate in the fact that the paper has been published in English, and since many of the organisms with which it deals are pathogens, it will be of interest to plant pathologists as well. The greater part of the article was written before the author had seen Whetzel's Synopsis of the Sclerotinaceae published in MYCOLOGIA in 1945; however, numerous footnotes referring to details of the MYCOLOGIA article afford a basis for comparison between Whetzel's and Buchwald's treatments of the family. Although differing in some respects, as might be expected, the two authors show a marked similarity of viewpoint toward the problems involved. *Stromatinia* Boud., recognized as a separate genus by Whetzel, is classified by Buchwald as a subgenus of *Ciboria*. Several species included by Whetzel in *Sclerotinia*, all stem-parasites on Cyperaceae and Juncaceae and possessing a *Myrioconium* conidial stage, were segregated by Buchwald in 1947 in his genus *Myriosclerotinia*. *Rutstroemia* is limited by Buchwald to species having pluricellular spores. Both authors advocate the recognition of the Sclerotiniaceae as a well-defined family, and propose the erection of the genus *Botryotinia* to include species having a *Botrytis* conidial stage.

The paper begins with a comprehensive presentation of the historical background of the Sclerotiniaceae, and a critical analysis of the earlier concepts held by Fuckel, Phillips, Rehm, Schroeter and Boudier, and the later work of Seaver, Nannfeldt, Honey, Weiss, White and Whetzel. In connection with the discomycete genus *Botryotinia*, an excellent review of the genus *Botrytis* is given and

its relationship to the Sclerotiniaceae discussed. The generic diagnosis of *Botrytis* is emended and three subgenera proposed. *Ciboria* is redefined and the eighteen recognized species divided into two subgenera. Specific names are given to hitherto unnamed *Botrytis* stages of various *Botryotinia* species, also to the *Myrioconium* and *Sclerotium* stages of some species of *Myriosclerotinia*. The conidial stage of *Septotinia* is named as *Septotis*, and that of *Ovulinia* as *Ovulitis*. In his diagnosis of the Sclerotiniaceae, the author stresses as the two most important characters: the development of apothecia from a more or less well-developed stroma or sclerotium, and the microconidial or spermatial stage, common to fungi in this family but not found in related Discomycetes. A key to genera, subgenera, and sections concludes the paper. The evident completeness and accuracy of data and the clearness of presentation combine to make this a valuable addition to the literature on the subject.—EDITH K. CASH.

PATHOLOGY IN FOREST PRACTICE, by Dow Vawter Baxter. Second edition. xiii + 601 p., 224 figs. John Wiley & Sons, New York. 1952. Price, $7.50.

This widely used text has been extensively revised, partly rewritten and set in new type. Many of the illustrations in the first edition have been taken out, and in most cases replaced by new and better ones, and a number of new cuts have been added. The chapter headings of the older edition have been retained, but the text of most of the chapters has been made more concise. Chapter IX, on handling and treatment of forest products, has been substantially expanded. The treatment of forest diseases incorporates knowledge which has come to light since the earlier edition was published, including reference to several diseases not known or not well understood at that time. The reference to chemical treatments has been revised in accordance with recent findings in this currently active field. The importance of considering the reactions between trees and their environment in attempting to explain the incidence of disease is emphasized. A minor defect is the failure to comply with modern nomenclatural practice in citing names of pathogenic

fungi. It is possible, of course, that it was felt that an attempt to do such consistently might result in more confusion than help to many users of the book.—G. W. M.

A RECORD OF THE FUNGI NAMED BY J. B. ELLIS. Compiled by Edith K. Cash. Special Publication issued by Div. of Mycology and Disease Survey, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. D. A. Part I. *Acanthostigma-Gymnosporangium*. 165 pp. Plant Industry Station, Beltsville, Maryland. 1952.

Within the alphabetical range covered, this list attempts to cite all names validly published by Ellis, as well as *nomina nuda* and unpublished herbarium names. The more important synonyms are added and reference is made to exsiccati issued by Ellis. It is expected that an additional part will appear during the coming year and that eventually the record will be completed.

This work will be of very great importance to all who are concerned in the taxonomy of fungi and should be available in connection with every extensive collection of fungi. Copies are still available for those who have not received them and may be secured on request, so long as the supply lasts, by writing the Division.—G. W. M.

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